# 2-Alkoxycarbonyl Allyl Ester Conjugates of NSAIDs as Potential Anticancer Agents

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Erica Lueth

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Venkatram Mereddy

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**Abstract**

Enzyme cyclooxygenase (COX) inhibition with non-steroidal anti-inflammatory drugs (NSAIDs) has long been utilized to treat inflammation and relieve pain. Several studies have shown that NSAIDs have also cancer preventative and tumor regressive effects. Prostaglandin $E_2$ which acts as an inflammatory mediator influences many mechanisms that plays a significant role in tumorigenesis such as cell proliferation, angiogenesis, and metastasis. COX overexpression is a characteristic feature of most malignant tumors and contributes to poor outcomes in multiple malignancies. It has been reported that cancer incidence can be reduced by 25-40% in patients regularly taking low dose COX inhibitor aspirin on a daily basis, with the most compelling evidence acquired for colorectal cancer. We envisioned that NSAID conjugates derived from 2-alkoxycarbonyl allyl esters would have cytotoxicity enhancing prodrug properties with dual anti-inflammatory and intracellular alkylation. In the current work, 2-alkoxycarbonyl allyl ester conjugates of several common NSAIDs have been synthesized and tested for their cell proliferation inhibition properties in breast (MDA-MB-231, 4T1), pancreatic (MIA PaCa-2), and colorectal adenocarcinoma (WiDr) cell lines. Several of the synthesized derivatives exhibit good potency against all four cancer cell lines. The synthesized compounds have also been tested for their COX inhibition properties.

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| | |
|---|---|
| NSAID | Non-Steroidal Anti Inflammatory Drug |
| COX | Cyclooxygenase |
| PGE2 | Prostaglandin |
| $CdCl_2$ | Cadmium chloride |
| MEF | Mefenamic acid |
| $IC_{50}$ | half maximal inhibitory concentration |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| CDI | 1'-Carbonyldiimidazole |
| RGD | |
| EPB | Epirubicin |
| HBTU | (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) |
| DIPEA | Diisopropylethylamine |
| PIA | phospho-ibuprofen amide |
| DMAP | 4-Dimethylaminopyridine |
| DCC | N,N'-Dicyclohexylcarbodiimide |
| ADT | anethole dithiolethione |

| | |
|---|---|
| EDC | 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide |
| PyBOP | benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate |
| $POCl_3$ | Phosphoryl chloride |
| TEA | Triethylamine |
| THF | Tetrohydrofuran |
| $CH_3OH$ | Methanol |
| NaOH | Sodium hydroxide |
| LAH | Lithium aluminum hydride |
| Pd/C | Palladium on carbon |
| DMF | Dimethylformamide |
| BH | Baylis-Hillman |
| DABCO | 1,4-diazabicyclo[2.2.2]octane |
| HBr | Hydrobromic acid |
| $H_2SO_4$ | Sulfuric acid |
| SAR | Structure activity relationship |
| DMSO | Dimethyl sulfoxide |
| $K_2CO_3$ | Potassium carbonate |
| EtOAc | Ethyl acetate |

| | |
|---|---|
| NMO | N-methylmorpholine-N-oxide |
| | |

# CHAPTER 1: INTRODUCTION

NSAIDs have recently been exploited for having a second purpose in the body besides anti-inflammatory properties. There are many cancers that are related to chronic inflammatory conditions such as the link between inflammatory bowel disease and colon cancer. There is strong evidence to suggest that taking an NSAID regularly can help decrease your risk of developing colon cancer; and in this regard NSAIDs have been exploited for their potential as anticancer agents.

In 2012, Hanahan and Weinberg identified several distinct hallmarks that lead to tumor growth (**Figure 1a**). Inflammation has been recognized as one of the important markers of cancer development and growth and consequently, developing anti-inflammatory agents that are specific towards cancer cells will be highly useful towards discovering novel anticancer agents.[4]

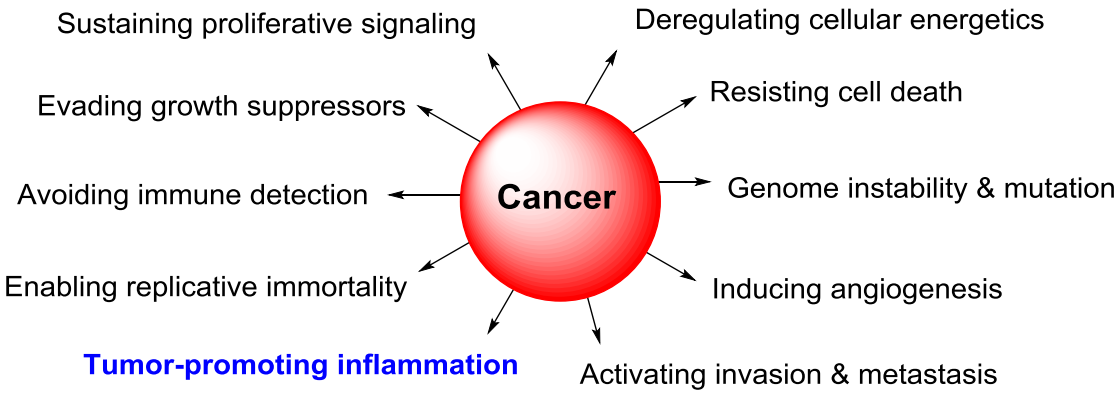



**Figure 1a:** Hallmarks of cancer

When experiencing inflammation, an enzyme called cyclooxygenase (COX) is involved. There are two types of COX enzymes, COX-1 and COX-2. COX-1 is present throughout normal and cancer cells. It is responsible for many biological pathways such as helping to develop stomach lining and making platelets for the removal of clots. The second enzyme, COX-2, is

only present when there is an inflammation. It is no surprise that tumors being inflammatory in nature also overexpress COX-2 enzyme. Many NSAIDs are not able to distinguish between inhibiting COX-1 or COX-2, which is why most NSAIDs have gastrointestinal side effects.

COX-2 is commonly found in premalignant lesions, carcinoma *in situ*, invasive cancer, and metastatic disease. COX-2 is responsible for forming prostaglandin (PGE2), and this upregulation can be found responsible for many different pathways in tumorigenesis including immunosuppression, mutagenesis, anti-apoptosis, metastasis, mitogenesis, and angiogenesis (**Figure 1b**).

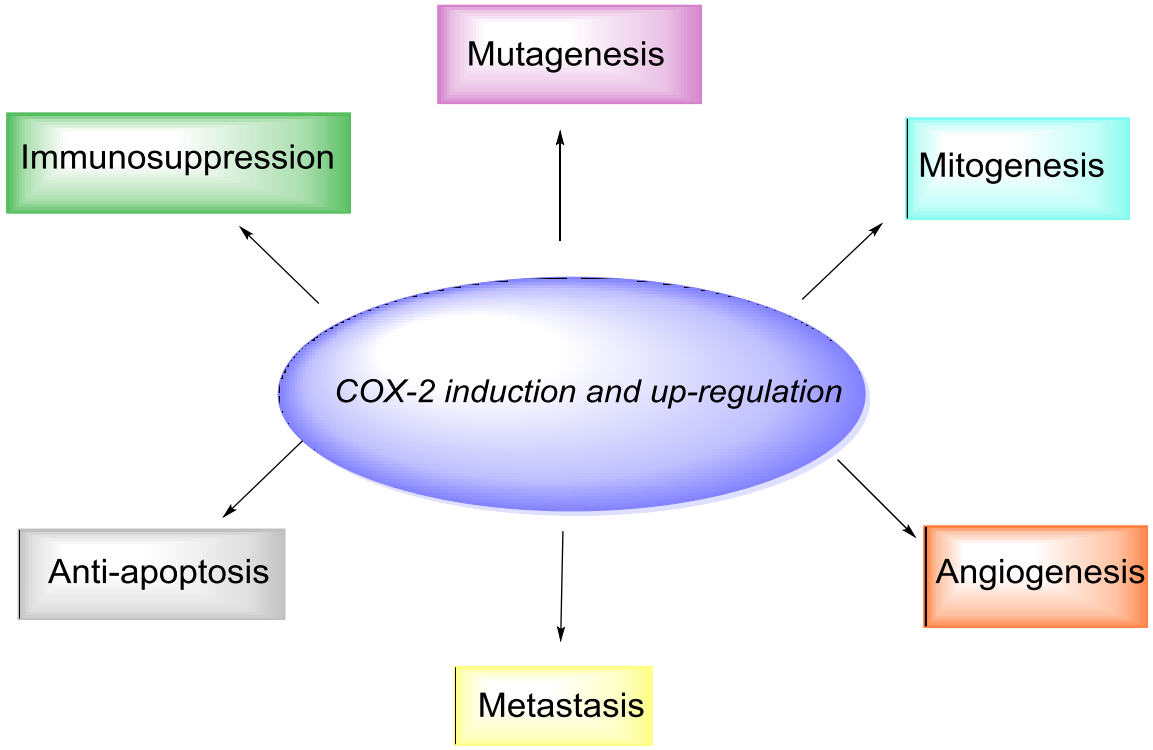



**Figure 1b:** Role of COX-2 in cancer development

Some of the commonly used NSAIDs for various pain related ailments are shown in **Figure 1c**.

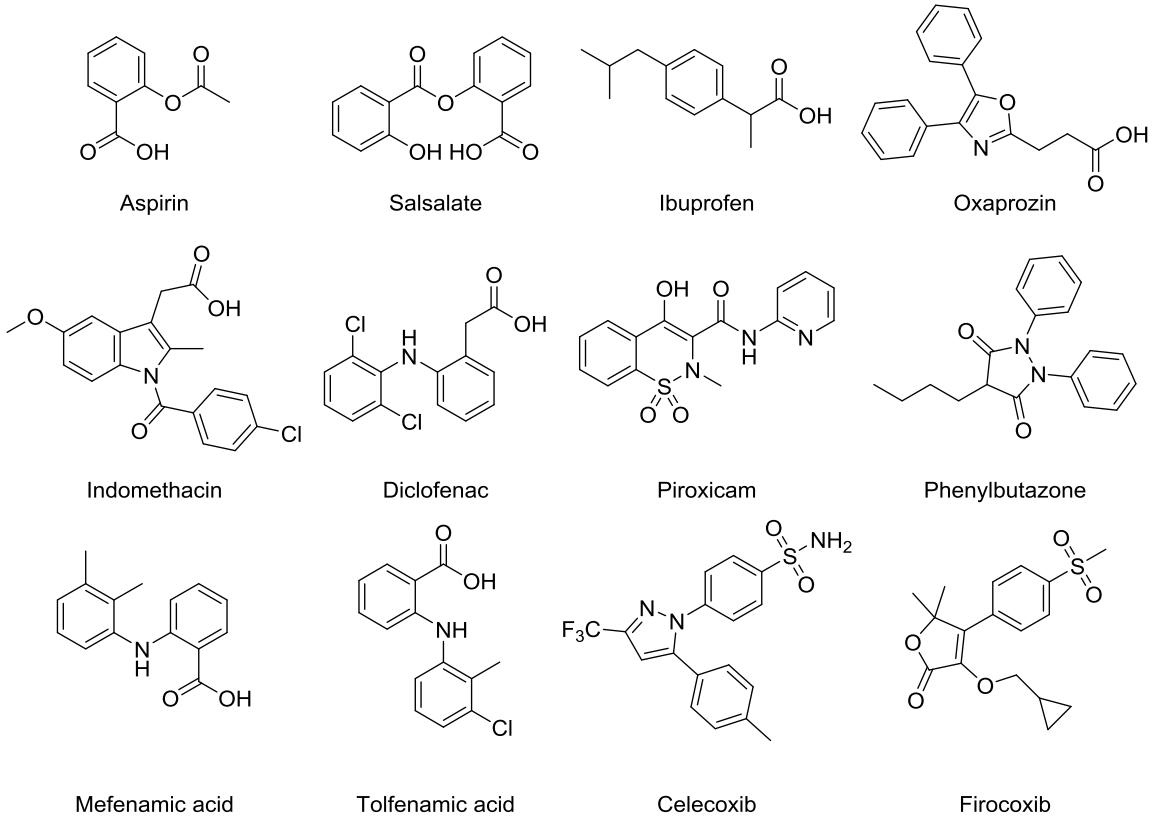
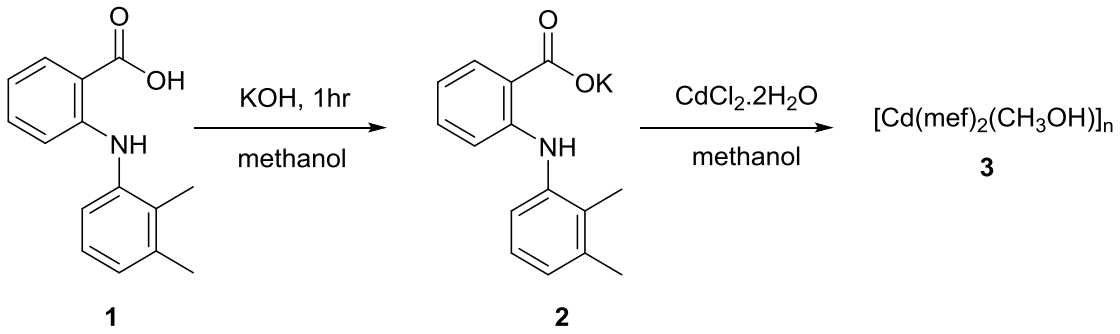



**Figure 1c:** Commonly used NSAIDs

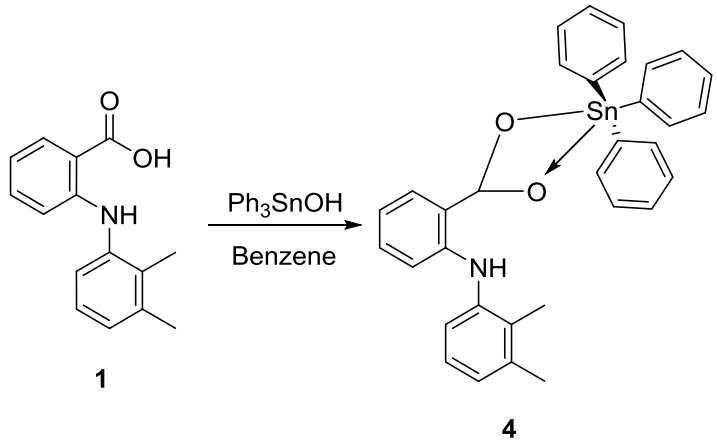
Owing to the importance of cyclooxygenase in cancer progression, there have been several synthetic and biological studies for the development of novel NSAID based cyclooxygenase inhibitors as anticancer agents. The following are few representative synthetic methods that have been reported for the preparation of NSAID based anticancer agents.

**Metallo derivatives of NSAIDs**

Tabrizi *et al.* synthesized mefenamic acid potassium salt **2** from NSAID mefenamic acid **1**. The salt was added to a methanolic solution of $CdCl_2$ to provide the complex $[Cd(mef)_2(CH_3OH)]_n$ **3** (**Scheme 1.1**). It exhibited an $IC_{50}$ value of 0.12±0.56 µM against MCF-7 breast cancer cell line and 0.86±0.21 µM against T-24 bladder cancer cell line.[6]



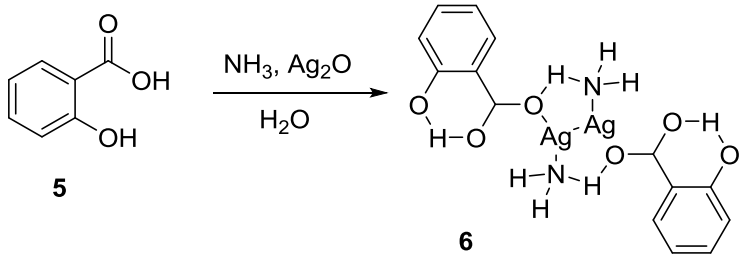

**Scheme 1.1:** Synthesis of mefenamic acid cadmium complexes[6]




**Scheme 1.2:** Synthesis of NSAID triphenyltin(VI) complexes[7]

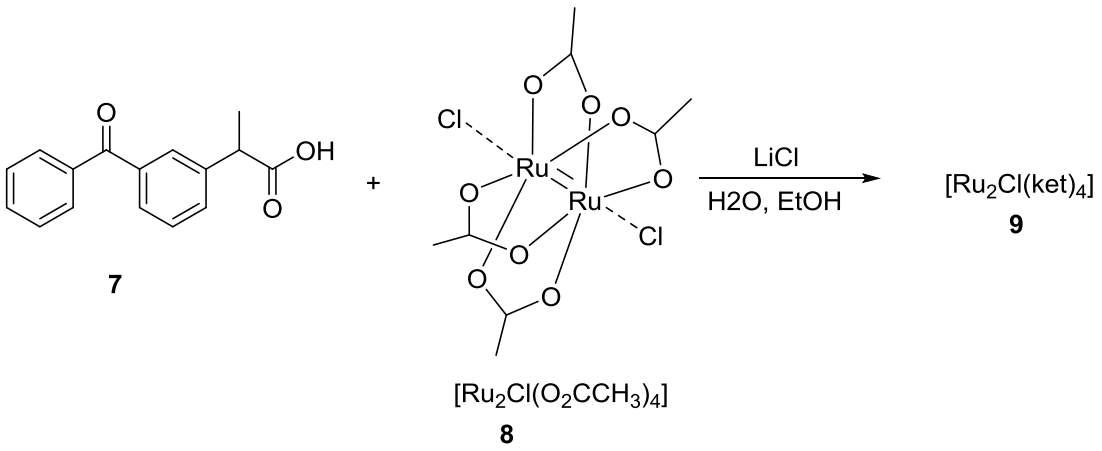
Coyle *et al.* reported the reaction of NSAID salicylic acid **5** with silver oxide to form dimeric Ag(I) complexes (**Scheme 1.3**). These derivatives exhibited both antifungal and anticancer properties. The complex **6** was tested on squamous carcinoma of tongue Cal-27,

hepatocellular carcinoma Hep-G2 and kidney adenocarcinoma A-496 cell lines using an MTT assay. The compound exhibited cell proliferation inhibition of 51, 9, and 32 μM respectively.[8]



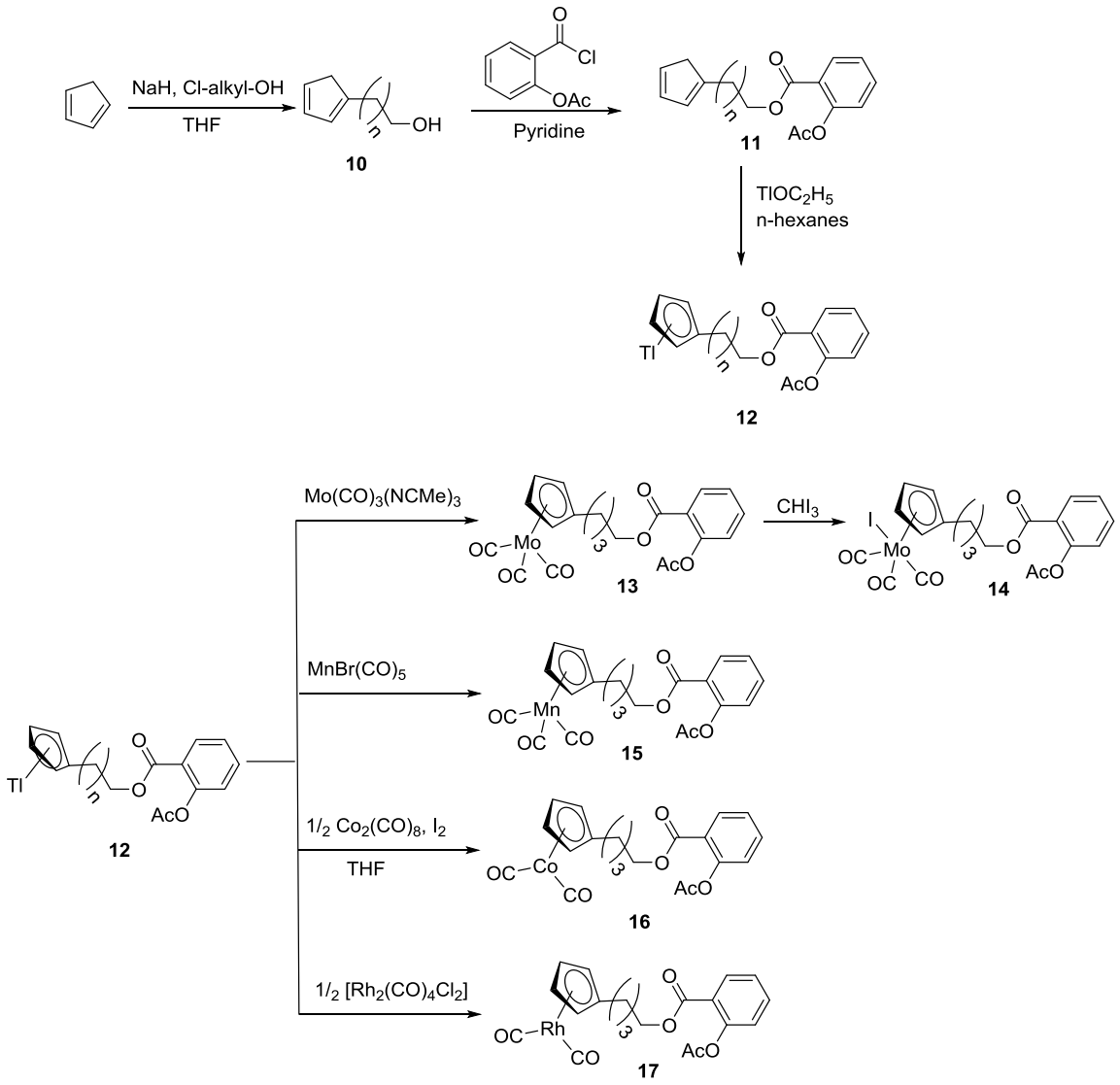

**Scheme 1.3:** Synthesis of dimeric Ag(I) complexes of salicylic acid[8]

Rodrigo *et al*. synthesized a diruthenium(I,II)-ketoprofen complex using ketoprofen **7** and $[Ru_2Cl(O_2CCH_3)_4]$ **8** in the presence of lithium chloride (**Scheme 1.4**). This complex **9** was tested against COX-2 overexpressing HT-29 cell line and Caco-2 cell line with low COX-2 levels. Cytotoxicity profile of this complex in these two cell lines did exhibit low cell proliferation inhibition, signifying that COX-2 inhibition by these drugs did not affect the cytotoxicity.[9]




**Scheme 1.4:** Synthesis of diruthenium(II,III)–ketoprofen complex from ketoprofen[9]

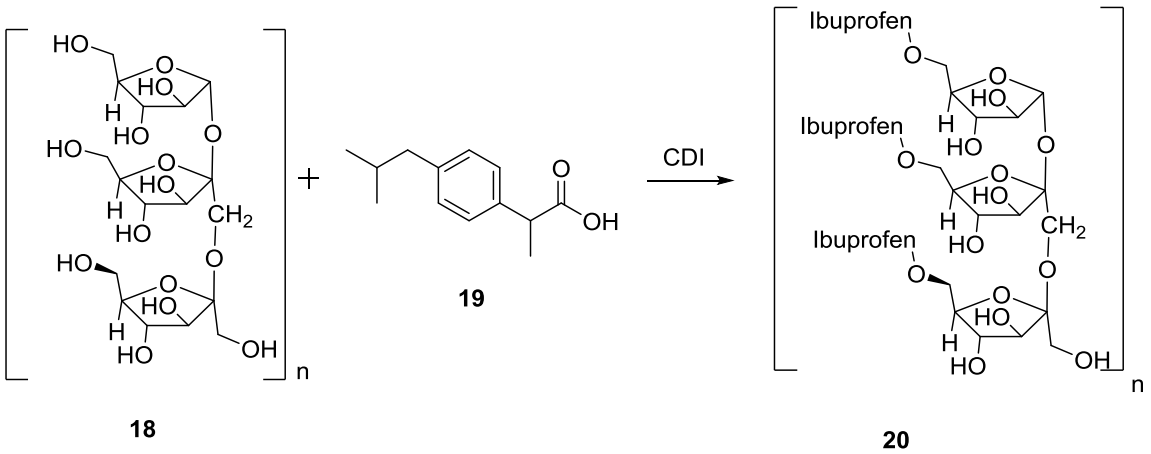
Gust *et al*. synthesized several [cyclopentadienyl]metalcarbonyl complexes of acetylsalicylic acid. Cyclopentadienyl alcohols **10** were prepared by the reaction of cyclopentadiene with sodium hydride followed by the addition of alcohol. The alcohol was coupled with acetylsalicylic acid chloride in the presence of pyridine to form (cyclopentadienyl)alkyl-2-acetoxybenzoate **11**. This derivative was further treated with thallium ethoxide to obtain $\eta^5$-[(cyclopentadienyl)alkyl-2-acetoxybenzoate]thallium **12**. This complex was further functionalized into various metal complexes **13-17** (**Scheme 1.5**). These complexes were evaluated for COX-2 enzyme inhibition, and prop-Cp-ASS-metal complexes **13-17** were found to have more inhibitory effects compared to the parent aspirin. The complexes **13-17** were also tested for their cytotoxicity in MCF-7 and MDA-MB-231 breast cancer cells and HT-29 colon cancer cells. **12** exhibited high cytotoxicity in the range of 1.4 to >50 uM in MCF-7, 1.9 to >50 uM in MDA-MB-231 and 4.6 to >50 µM in HT-39 cells.[10]




**Scheme 1.5:** Synthesis of [cyclopentadienyl]metalcarbonyl complexes of acetylsalicylic acid[10]

**Non-metallo derivatives of NSAIDS**

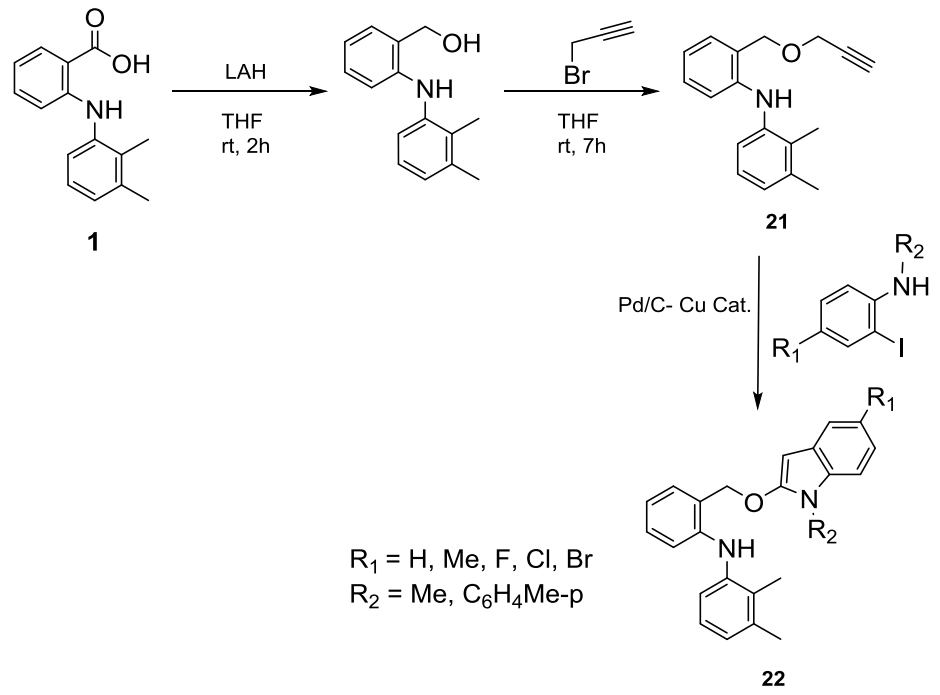
Inulin **18** was coupled with ibuprofen **19** in the presence of CDI to synthesize nanoparticalized derivative for targeted delivery of epirubicin, an anticancer drug. RGD conjugated EPB loaded nanoparticles were then used to encapsulate the inulin-ibuprofen polymer **20** (**Scheme 1.6**). These nanoparticles were tested against BGC832 cell line and were found to have relatively non-cytotoxic compared to the parent drug epirubicin. They were also evaluated for their anticancer efficacy in H22 xenograft model in male ICR mice and were found to have increased tumor growth inhibition compared to free epirubicin.[11]




**Scheme 1.6:** Synthesis of inulin-ibuprofen polymer[11]

Babu *et al.* synthesized mefenamic acid based novel indole analogues starting from indole derivatives. Mefenamic acid in **1** was reduced with lithium aluminum hydride followed by alkylation with propargyl bromide to give the corresponding alkylated product **21**. Treatment of **21** with o-iodoaniline in the presence of copper catalyst and palladium-carbon provided the indolyl mefenamic acid derivative **22** (**Scheme 1.7**). These compounds were then tested for their

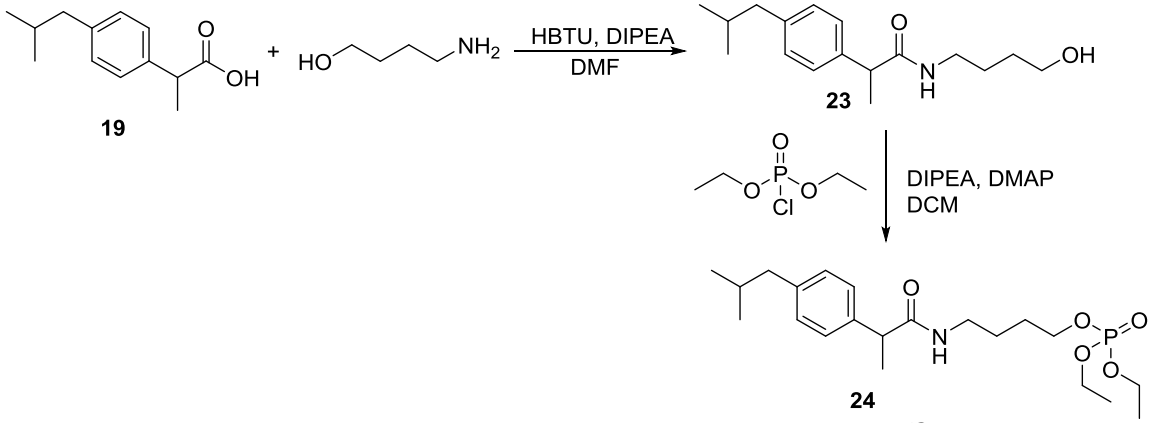
anticancer properties on breast cancer cell line MCF-7 to provide an $IC_{50}$ ranging from 0.75-10 μM.[12]




**Scheme 1.7:** Synthesis of mefenamic acid indole derivatives[12]

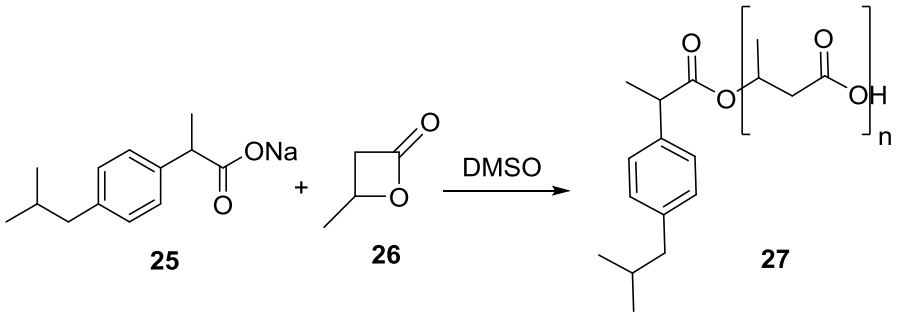
Rigas *et al*. synthesized phospho-ibuprofen amides to increase the stability of phopho-ibuprofen derivatives. Ibuprofen **19** was coupled to 4-aminobutanol in the presence of HBTU and diisopropylethylamine (DIPEA) to obtain N-(4-hydroxybutyl)-2-(4-isobutylphenyl)propenamide **23**. This alcohol **23** was converted into phospho-ibuprofen amide (PIA) **24** by reacting the alcohol with diethylchlorophosphate in the presence of DIPEA and DMAP (**Scheme 1.8**). This derivative PIA was further nanoparticalized by encapsulating in liposomes. This liposome encapsulated PIA was tested against several non-small cell lung carcinoma cell lines and it was found to be 10 times more potent than ibuprofen. Pharmacokinetic studies of this PIA showed that this form exhibits metabolic stability against

hydrolysis with carboxylesterases. Liposome encapsulated PIA also exhibited significant tumor growth inhibition compared to ibuprofen in A549 xenograft model.[13]




**Scheme 1.8:** Synthesis of phospho-ibuprofen amides[13]

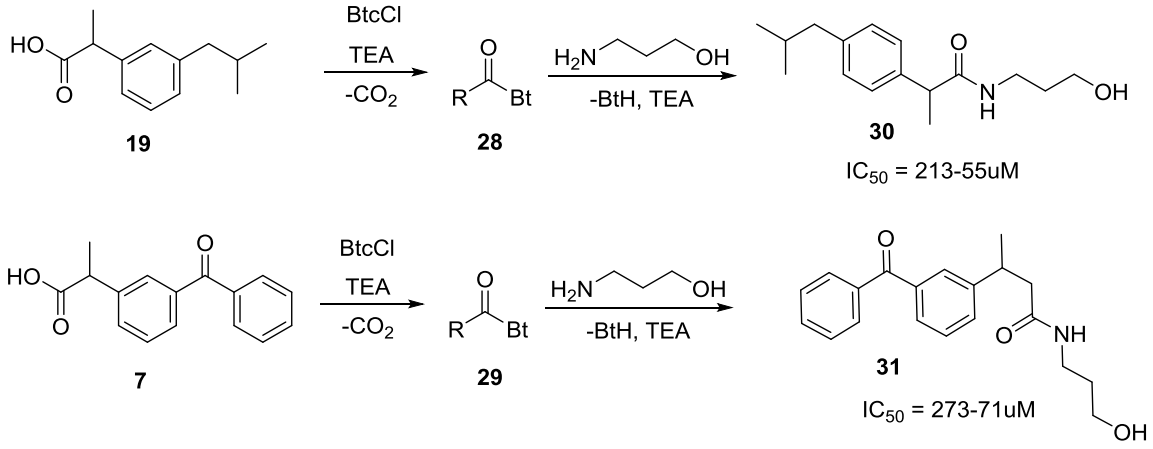
Zawidlak-Węgrzyńska, *et al.* synthesized novel conjugates of oligo(3-hydroxybutyrate) and ibuprofen. The ibuprofen was made into a sodium salt **25** and then reacted with the oligo(3-hydroxybutyrate) **26** to give the oligomers of ibuprofen **27**. This was further tested for anticancer properties on two colon cancer cell lines, HT-29 and HCT 116, using an MTT assay. These $IC_{50}$ values ranged from 37-77 µM in the HT-29 line and 31-53µM in the HCT 116 cell line.[14]




**Scheme 1.9:** Sythesis of Ibuprofen Oligo(3-hydroxybutyrate) Derivatives[14]

Wittine *et al.* conjugated NSAIDs ibuprofen **19** and ketoprofen **7** with 3-hydroxypropylamides. Ibuprofen and ketoprofen benzotriazolides **28** and **29** were reacted with 3-

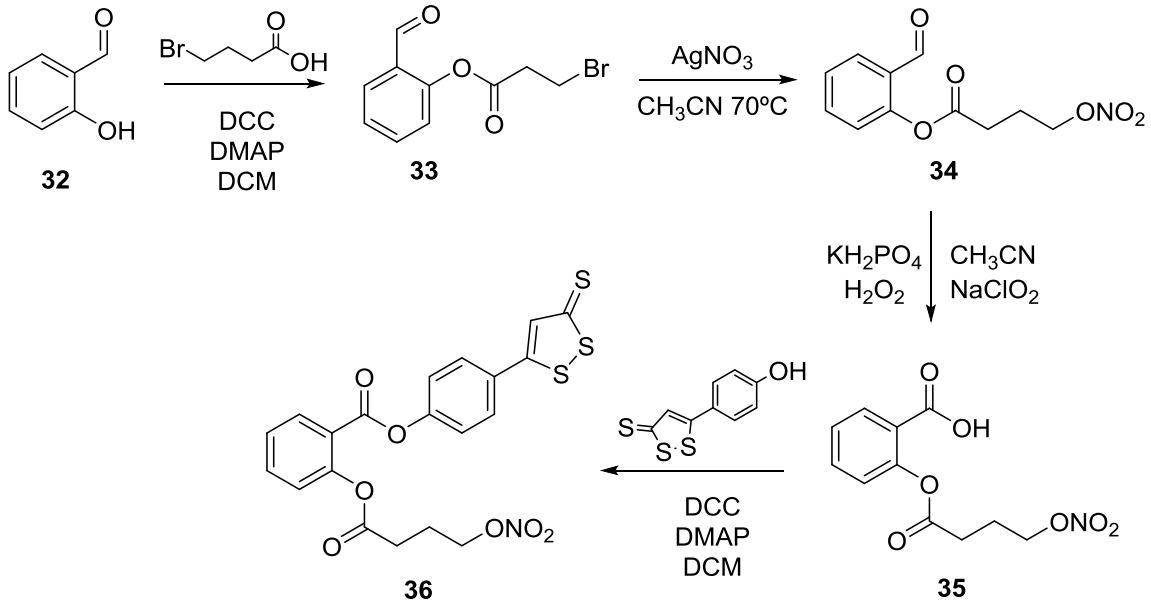
hydroxypropylamine in presence of trimethylamine to provide corresponding amide products **30** and **31** (**Scheme 1.10**). The compounds were then tested on nine different cell lines (L1200, Molt 4/C8, CEM, HeLa, MIA PaCa-2, SW 620, MCF-7, H 460, and WI 380). These compounds did not exhibit any significant cytotoxic properties and the $IC_{50}$ values ranged from 55-273 μM in cell lines L1200, Molt 4/C8, and CEM.[15]




**Scheme 1.10:** Synthesis of ketoprofen and ibuprofen 3-hydroxypropylamide derivatives[15]

Vannini *et al.* synthesized NOSH-aspirin, a molecule capable of releasing hydrogen

sulfide and nitric oxide intracellularly. 2-hydroxybenzaldehyde **32** was coupled with 4-bromobutanoic acid in the presence of DCC and catalytic DMAP to obtain bromo derivative **33.** This bromide undergoes $S_N2$ type reaction with silver nitrate to get the O-nitrated compound **34**. The aldehyde **34** was oxidized to carboxylic acid using Pinnick oxidation protocol. The carboxylic acid **35** was further coupled with ADT-OH to give the desired NOSH-NSAID product **36** (**Scheme 1.11**). The compound was shown to have an $IC_{50}$ of 48 µM on colon cancer line HT-29 and 57 µM on HCT 15.[16]

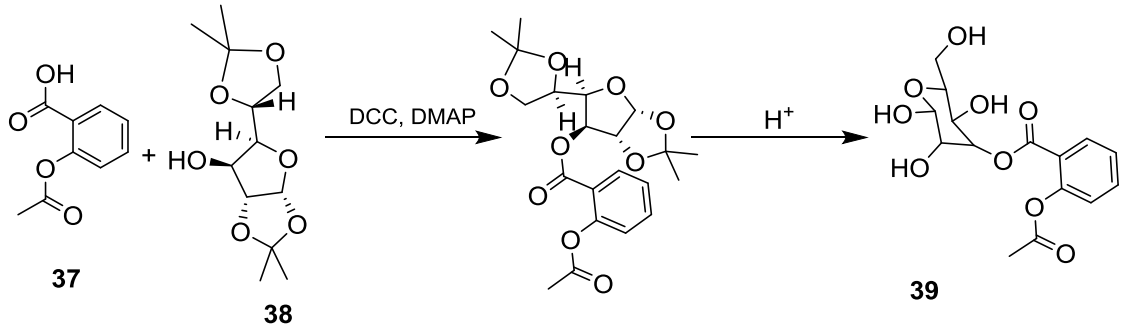



**32** **33** **34** **35** **36**

**Scheme 1.11:** Synthesis of NOSH-aspirin derivative[16]

Jacob and Tazawa synthesized an aspirin-glucose derivative by coupling aspirin **37** with dimethyl acetal protected glucose **38** using DCC as a coupling agent. The dimethyl acetal

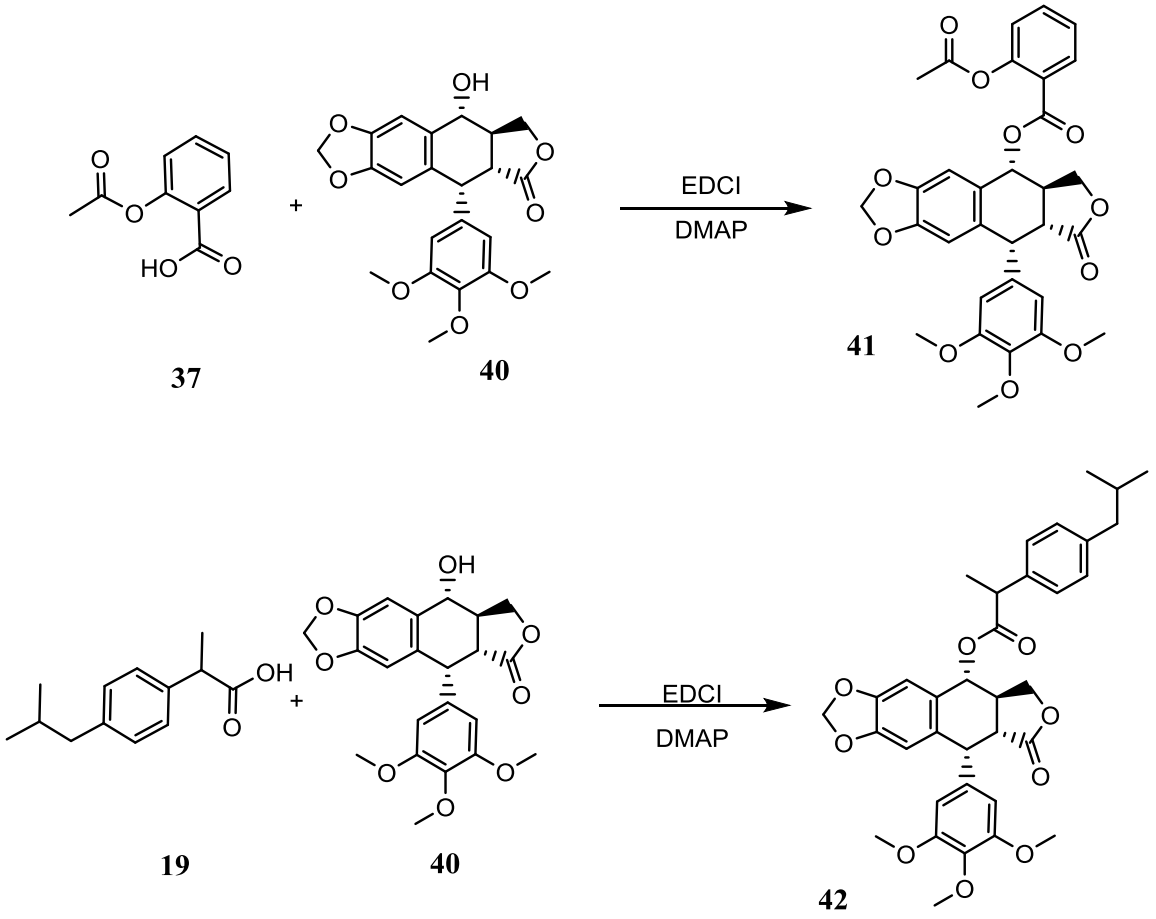
protecting group was deprotected under acidic conditions to give the glucose aspirin conjugate **39** (**Scheme 1.12**). This derivative was then tested on three cancer cell lines breast, pancreatic, prostate (SKBR3, PC3, PANC-1). The $IC_{50}$ for this compound ranged from 200-800 µM.[17]




**Scheme 1.12:** Synthesis of glucose-aspirin conjugate[17]

Zhang *et al.* synthesized cytotoxic podophyllotoxin-NSAID conjugates of aspirin and ibuprofen. The carboxylic acid group aspirin **37** or ibuprofen **19** were coupled with hydroxyl group in podophyllotoxin **40** with EDC in the presence of DMAP as a catalyst resulting in the products **41** and **42** (**Scheme 1.13**). These derivatives were tested for an $IC_{50}$ in three cell lines, Bel-7402, Bel-7402/5-FU and L-O2. The aspirin derivative had $IC_{50}$ values of 0.09, 0.065 and

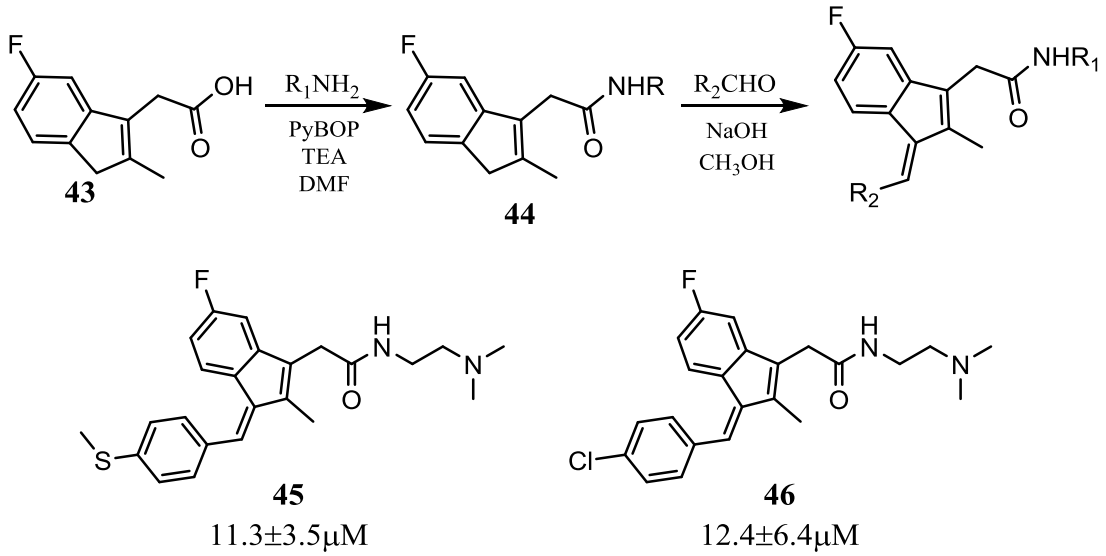
0.19 µM respectively; the ibuprofen podophyllotoxin derivative had $IC_{50}$ values in the 18.88, 10.27, and 7.38 µM range.[18]




**Scheme 1.13:** Synthesis of podophyllotoxin-NSAID derivatives with aspirin and ibuprofen[18]

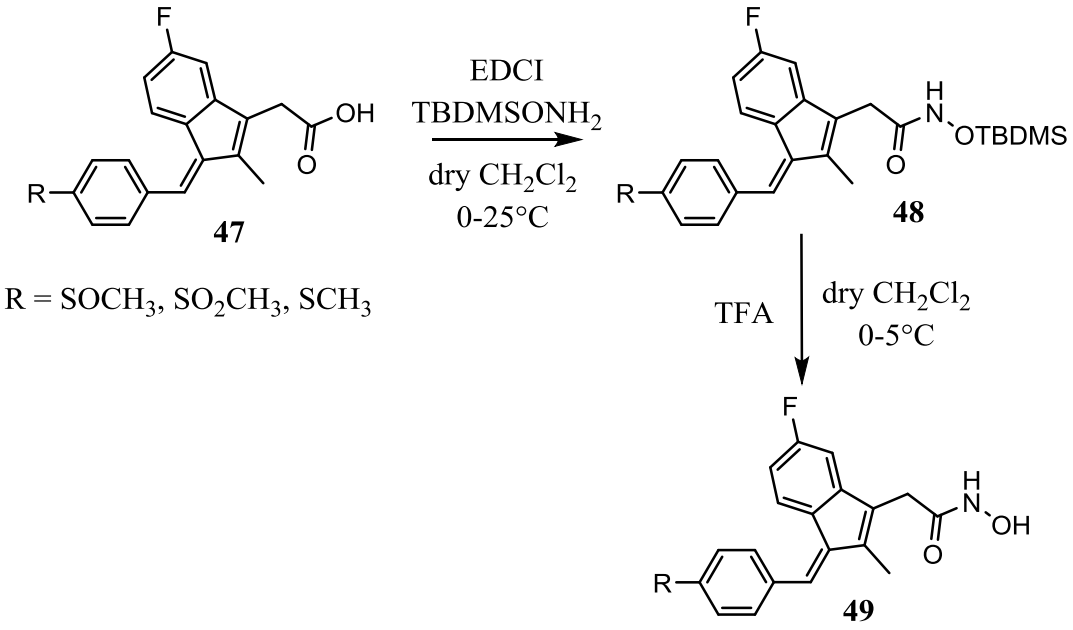
Chennamaneni *et al.* synthesized several derivatives of sulindac for the treatment of cancer. 2-(5-fluoro-2-methyl-1H-inden- 3-yl)acetic acid **43** was coupled with an amine in the presence of PyBOP and base. The resulting amide **44** was further reacted with an aldehyde in the presence of a base to give the target sulindac amides **45** and **46** (**Scheme 1.14**). The cytotoxic effects were tested on HT29 colon cancer cells. The most promising derivatives were the 4-

methylsulfinyl derivative **45** and the 4-chloro derivative **46** with $IC_{50}$ values of 11.3 and 12.4 μM, respectively.[19]



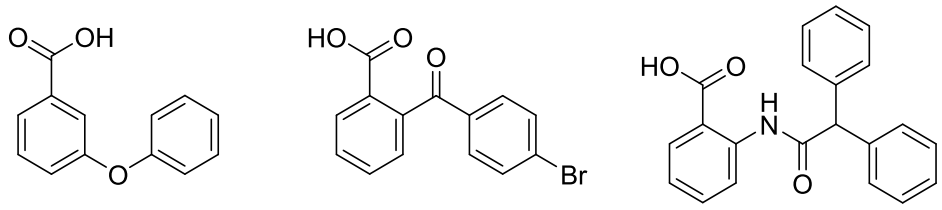

**Scheme 1.14:** Synthesis of sulindac derivatives[19]

Fogli *et al.* synthesized sulindac hydroxamic acid derivatives to see if substitution of the carboxylic acid group for hydroxamic acid could increase the toxicity of the derivative. (O-(tert-butyl-dimethylsilyl)hydroxylamine) was added to sulindac **47** in the presence of EDC to obtain O-silylated hydroxamates **48**. Trifluoroacetic acid deprotected the silyl group to give the product hydroxamic acid **49**. The $IC_{50}$ values of the derivatives ranged from 32-64 μM against MIA PaCa-2 cells, and provided $IC_{50}$ values ranged from 6-62.5 μM in COLO320 cells.[20]




**Scheme 1.15:** Synthesis of sulindac hydroxamic acid derivatives[20]

Gobec et al. synthesized NSAID analogs for AKR1C3 inhibition. AKR1C3 is a human enzyme that belongs to aldo-keto reductase superfamily. This enzyme is responsible for the conversion of androstenedione and estrone into testosterone and estrogen, respectively, in presence of NADPH cofactor. This enzyme plays a key role in the progression of hormone dependent breast and prostate cancers. Various NSAID analogs such as N-acylanthranilic acids, 2-benzoylbenzoic acids, benzophenones, and phenoxybenzoic acids **50-52** were synthesized and evaluated for their activity against human recombinant AKR1C3 (**Figure 1d)**. These analogs exhibited $IC_{50}$ values ranging from 0.68 to 180 μM.[21]



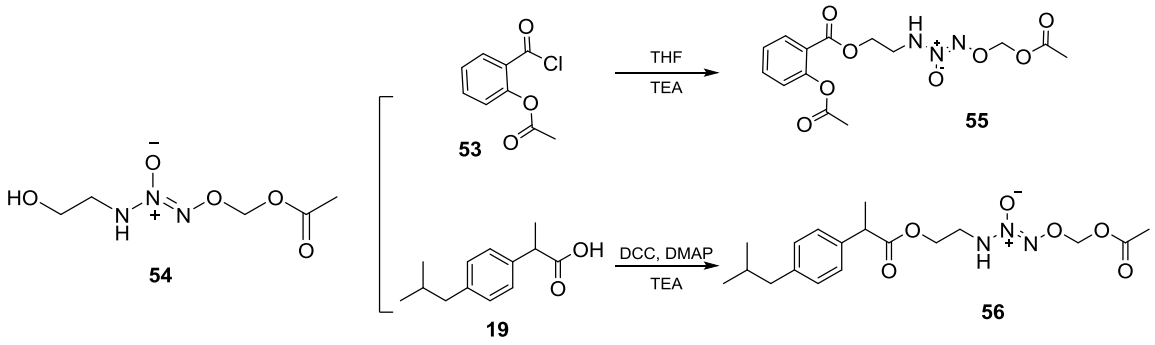

$IC_{50}$ = 0.68 μM **50**

$IC_{50}$ = 39 μM **51**

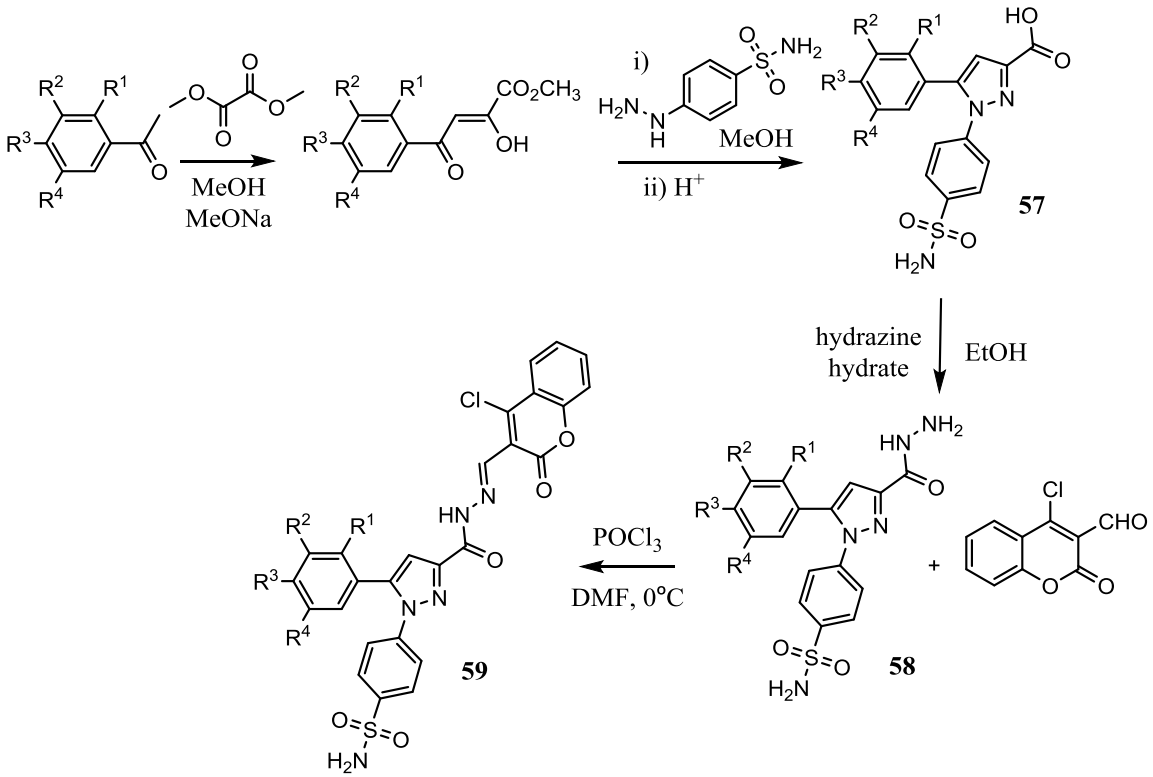
$IC_{50}$ = 11 μM **52**

**Figure 1d:** NSAID analogs for AKR1C3 inhibition[21]

Vela´zquez *et al.* synthesized novel NONO-NSAID derivatives using aspirin and ibuprofen. The acid chloride **53** and $O^{2-}$acetoxymethyl 1-[N-(2-hydroxyethyl)-N-methylamino]diazen-1-ium-1,2-diolate **54** were coupled together in triethylamine forming the desired product **55**. Ibuprofen **19** and $O^{2-}$acetoxymethyl 1-[N-(2-hydroxyethyl)-N-methylamino]diazen-1-ium-1,2-diolate **54** were coupled together in the presence of DCC and trimethylamine to give the product **56**. These compounds were tested for their inhibitory activity on COX-1 and COX-2 enzymes but did not show any noticeable effects.[22]



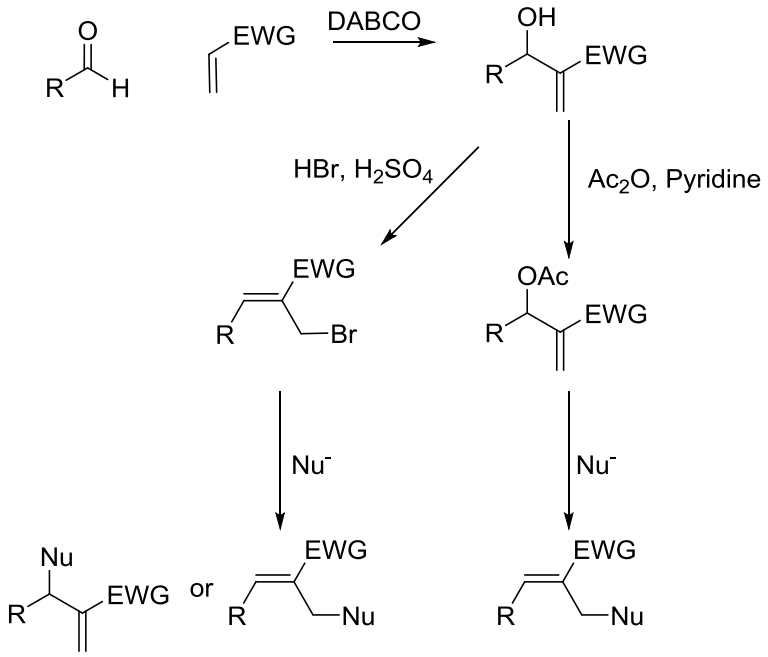

**Scheme 1.16:** Synthesis of NONO-aspirin and NONO-ibuprofen[22]Lu *et al.* synthesized celecoxib based coumarin sulfonamides. Various substituted chalcones were synthesized using dimethyl oxalate. These compounds were further coupled with 4-hydrazinylbenzenesulfonamide and hydrolyzed to obtain carboxylic acid **57**, which was treated with hydrazine hydrate to form the amides **58**. This was further functionalized to coumarin derivative **59** using $POCl_3$ (**Scheme 1.17**). All derivatives were tested against four cancer cell lines (HeLa, HepG2, F10, A549) and two non-cancer cell lines (293T, L02). The $IC_{50}$ values of compounds against HeLa cell lines were between 0.36 and 16.19μM and HepG2 cell lines were range from 0.85 to 21.19 μM. All compounds also exhibited less toxicity on the non-cancerous cell line than celecoxib.[23]




**Scheme 1.17:** Synthesis of celecoxib based coumarin sulfonamides[23]

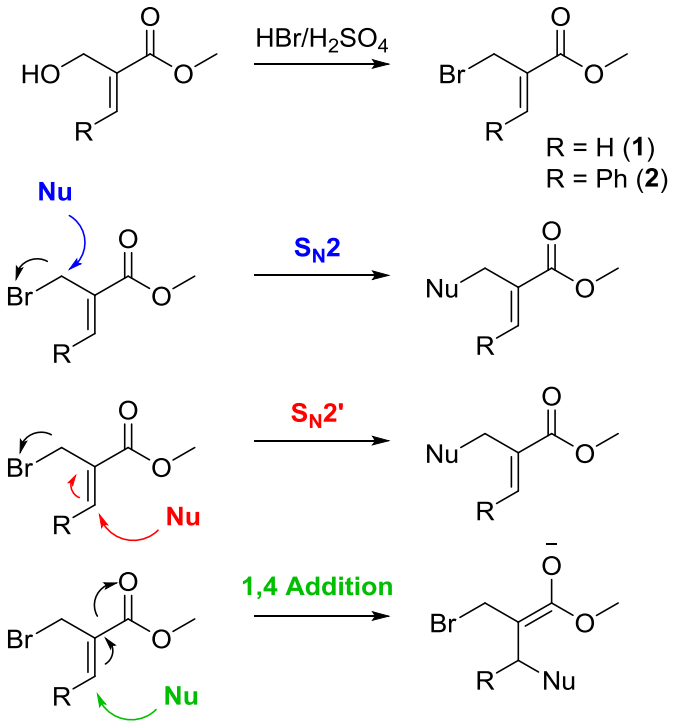
## CHAPTER 2: RESULTS AND DISCUSSION

Baylis-Hillman (BH) reaction is an important C-C bond forming reaction reported in organic chemistry. This reaction was originally discovered in the 1970s and it received wide attention in the last three decades as evidenced by thousands of scientific publications and numerous review articles.[24-28] This reaction can be easily carried out by simply mixing aldehydes/ aldimines/ activated ketones with α, β unsaturated esters, ketones, or nitriles, in the presence of a nucleophilic base such as DABCO (**Scheme 2.1**).[24-28] This reaction typically does not require usage of any solvents, heating, or any special reaction conditions. BH reaction provides good reaction yields, with complete atom economy. BH reaction also provides densely functionalized allyl alcohols and imines in one step. The product alcohols can be further functionalized by converting the alcohol into a leaving group such as an acetate or converting the alcohol into allyl bromide, followed by treatment with wide variety of nucleophiles containing C, O, S, N atoms etc (**Scheme 2.1**).[24-28]



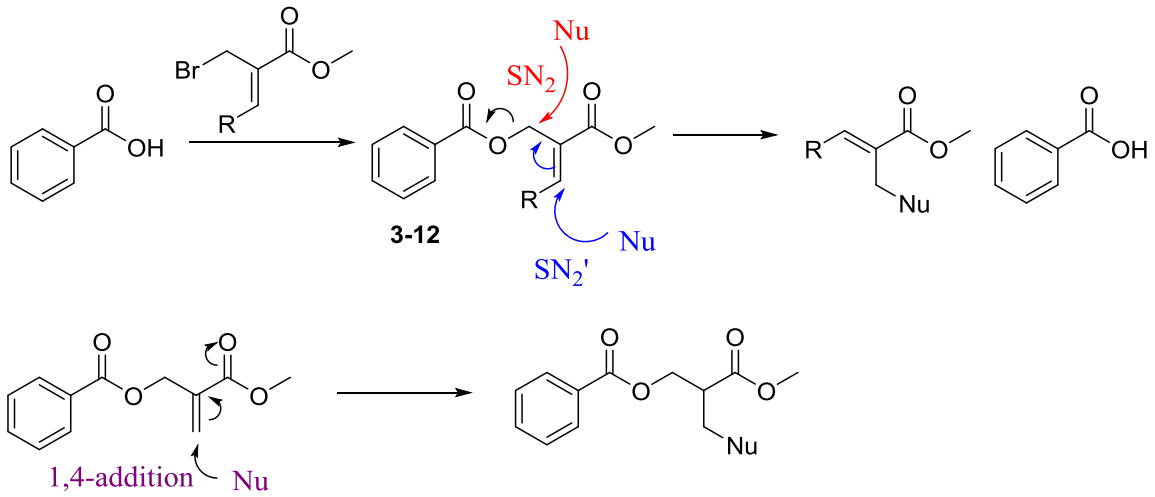

**Scheme 2.1:** General scheme of Baylis-Hillman reaction and nucleophilic isomerization

The allyl bromides **1** and **2** derived from BH alcohols are highly useful synthetic intermediates and our group has utilized these bromides for the synthesis of a wide variety of medicinally useful natural and synthetic products.[24-28] The allyl bromides **1** and **2** offer an interesting choice of reactive sites for the incoming nucleophiles, involving a direct $S_N2$ attack, $S_N2$' attack with allylic rearrangement, or a simple 1,4-addition to provide functionalized synthetic intermediates (**Scheme 2.2**).




**Scheme 2.2:** $S_N2$, $S_N2$' and 1,4-addition reactions of Baylis-Hillman bromides

Our long-standing interest in developing small molecule therapeutics using the BH reaction[29] has prompted us to utilize BH bromide **1** and **2** as a starting material. The bromides **1** and **2** are highly reactive and based on their structures, these bromides could act as DNA alkylating cytotoxic agents. However, these bromides suffer from serious drawbacks such as low chemical stability at room temperature, low metabolic stability, and high reactivity with the potential for serious side effects. We envisioned that α-carboxycarbonyl allyl esters **3-12** derived from carboxylic acids and BH bromides would retain the $S_N2$, or $S_N2$', or 1,4 addition properties with decreased reactivity and improved chemical stability to be developed as potential anticancer agents (**Scheme 2.3**). In this regard, recently, we carried out a study involving the synthesis of α-carboxycarbonyl allyl esters derived from various carboxylic acids.[29]




**Scheme 2.3:** Synthesis and $S_N2$, $S_N2$' and 1,4-addition reactions of α-carboxycarbonyl allyl esters

We have evaluated the cell proliferation inhibition studies of the synthesized compounds on several cancer cell lines. Our structure-activity relationship (SAR) study indicated that aromatic carboxylic compounds in general provided higher cell proliferation inhibition properties compared to aliphatic carboxylic acids. Introduction of electron donating or electron withdrawing group did not have much effect on the cell proliferation inhibition. Methoxy carbonyl group was found to be the optimal structural entity and removal of the double bond completely eliminated the biological activity, emphasizing the importance of $S_N2/S_N2$' mechanism. The $IC_{50}$ values of the α-carboxycarbonyl allyl esters **3-12** were found to be in the range from 3 - >100 µM against breast and pancreatic cancer cell lines, MDA-MB-231, 4T1, and MiaPaCa-2 (**Figure 2.1**).

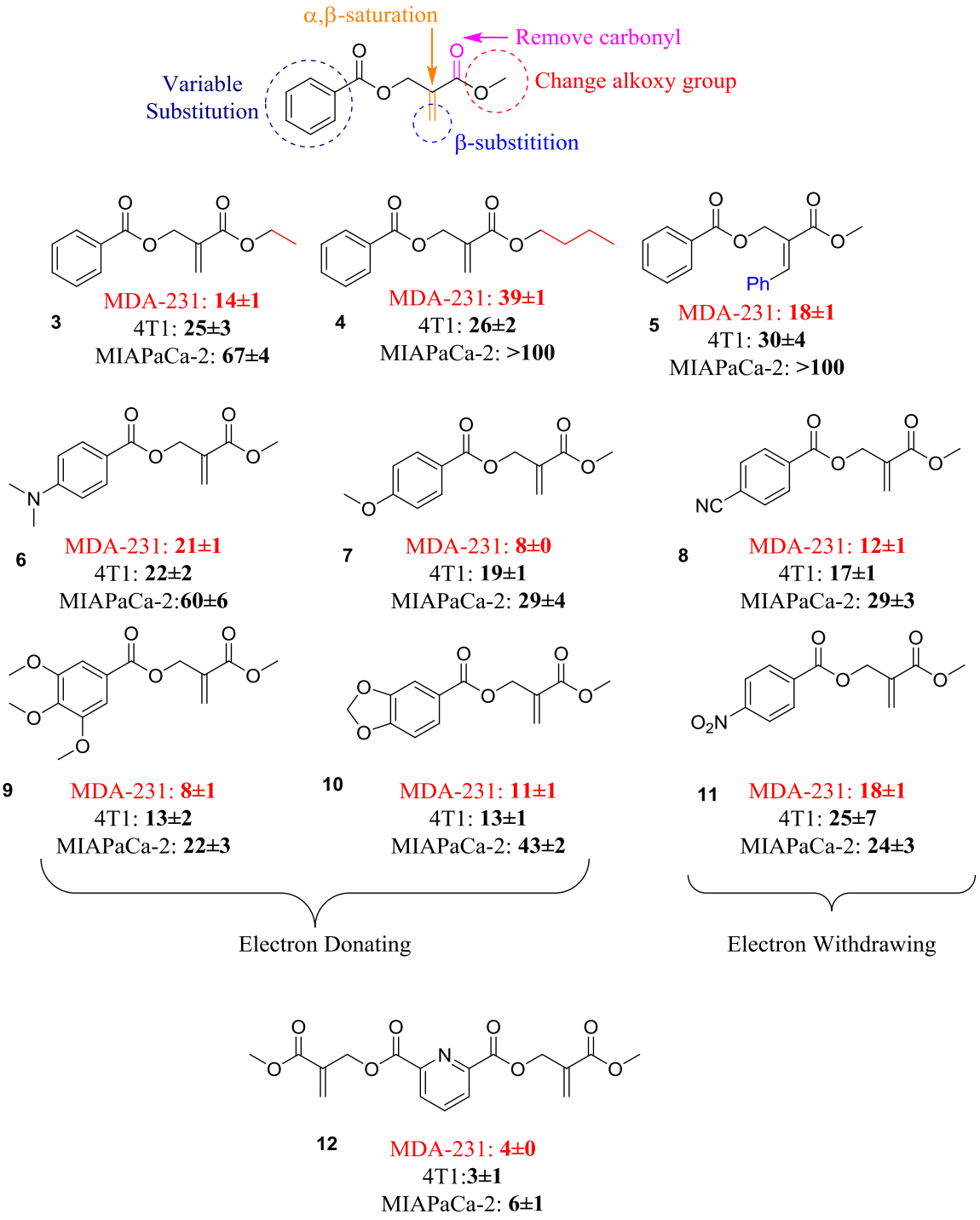



**Figure 2.1:** $IC_{50}$ values of various α-carboxycarbonyl allyl esters

The lead candidate compound **12** upon daily intraperitoneal administration was well tolerated in healthy CD-1 mice as evidenced by normal body weight gains compared to vehicle treated mice (**Figure 2.2A**). The lead candidate **12** also exhibited significant tumor growth inhibition in a triple negative breast cancer xenograft model MDA-MB-231 (**Figure 2.2**). [29]

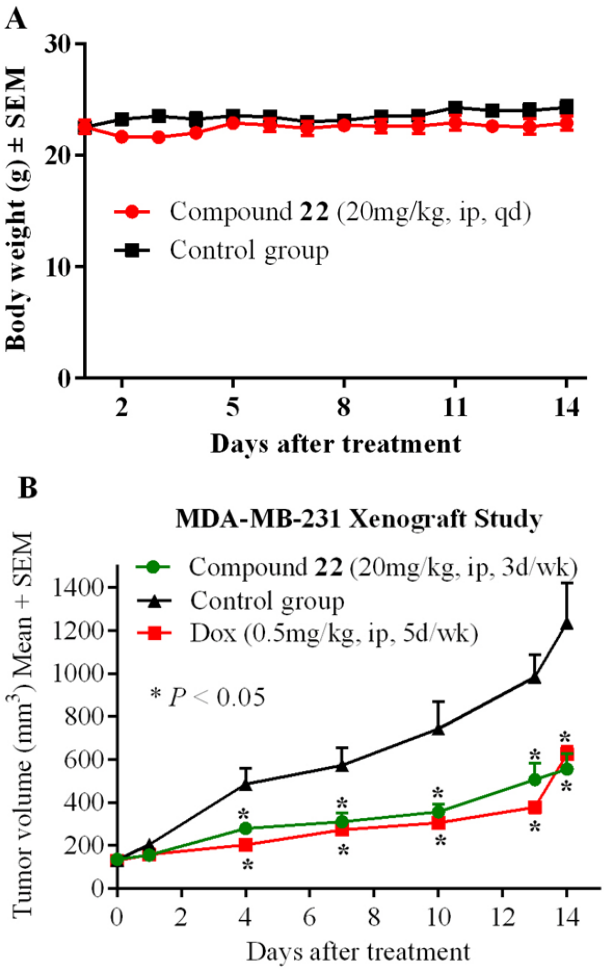



**Figure 2.2: A**) Systemic toxicity study of **12** in CD-1 mice; **B**) Anticancer efficacy study of **12** in MDA-MB-231 tumor xenograft model

As described in the introduction, inflammation has been recognized as one of the important tumor markers that can be targeted for anticancer efficacy in many solid tumors. Although several NSAIDs have been used as anticancer agents, and/or chemoproventative agents, the general lack of cytotoxicity precludes them to be used as primary chemotherapeutic

agents. In the present work, we hypothesize that conjugating the BH bromide with carboxy containing NSAIDs would lead to α-carboxycarbonyl allyl esters of NSAIDs with enhanced cytotoxic properties. These esters would also release the parent NSAIDs upon interacting with intracellular nucleophilic components either in $S_N2$ or $S_N2'$ fashion (**Figure 2.3**). We envisaged that these dual mechanistic properties should provide higher anticancer efficacy than using NSAIDs alone.

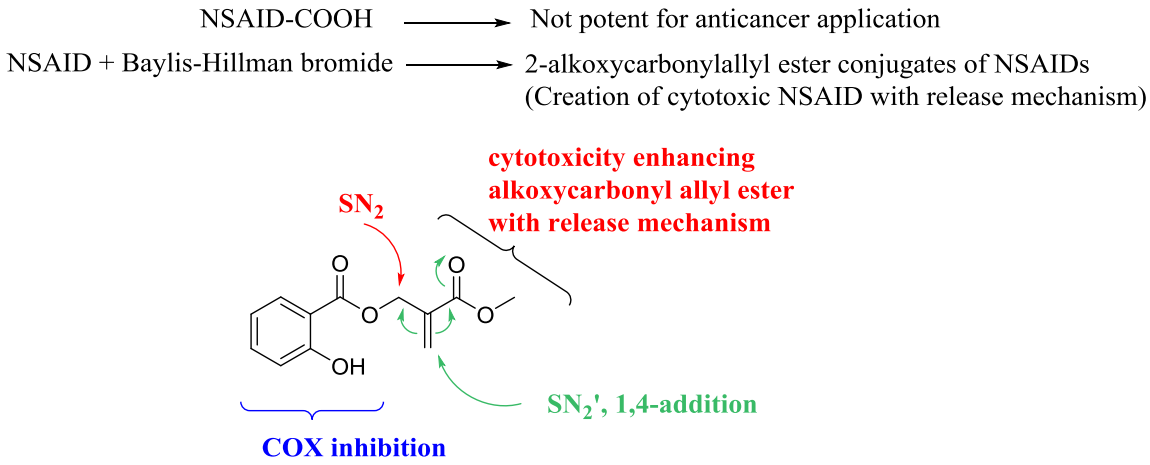
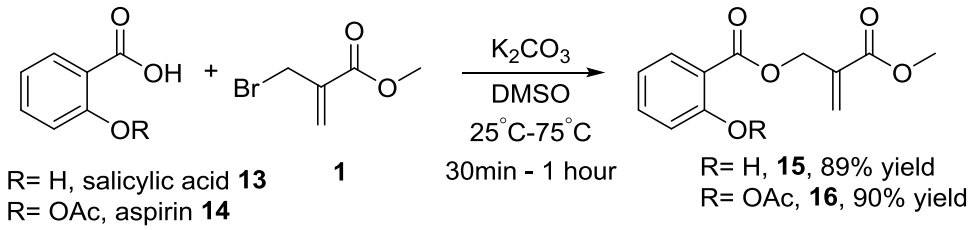


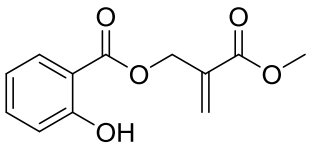

**Figure 2.3:** Hypothesis: Conjugates of NSAID carboxylic acids with BH bromides

With the above hypothesis in mind, initially we chose salicylic acid **13** for conjugating with BH bromide **1** derived from formaldehyde. The reaction of salicylic acid **13** with BH bromide **1** in the presence of potassium carbonate in DMSO provided methoxycarbonylallyl ester **15** (**Scheme 2.4**). The pure **15** could be readily obtained in 89% yield by silica gel column chromatography using 2% ethyl acetate in hexanes as eluent. Using the similar protocol, we also synthesized carboxycarbonyl allyl ester **16** from acetylsalicylic acid **14** (aspirin) in 90% yield.

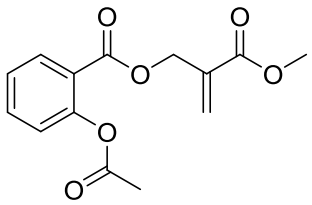



**Scheme 2.4:** Synthesis of carboxycarbonyl allyl esters from salicylic acid and aspirin

The carboxy esters **15** and **16** were evaluated against human triple negative breast cancer cell line MDA-MB-231 and highly metastatic murine breast cancer cell line 4T1, pancreatic cancer cell line MIAPaCa-2 and colorectal adenocarcinoma cell line WiDr. The cell proliferation inhibition studies were carried out using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. MTT is reduced to formazan through mitochondrial reductase with an absorbance at 590nm. Cell cultures in 96-well plates were incubated with the compounds for 72 h, and MTT values expressed as percent of vehicle-only (control) wells. The $IC_{50}$ value was calculated for each compound as the dose required to suppress the MTT signal to 50% of control values. These assays were carried out a minimum of three trials and the $IC_{50}$ values were calculated using GraphPad Prism 6 Software. Gratifyingly, the $IC_{50}$ values for **15** and **16** were found to be in the range from 4 - 17 µM for the above-mentioned cell lines (**Figure 2.4**). It is important to note here that the parent NSAIDs salicylic acid **13** and acetylsalicylic acid **14** did not show any cell proliferation inhibition against any of the cell lines that we used even at 100 µM concentration.



MIAPaCa-2: 15±1 μM
MDA-MB-231: 17±2 μM
4T1: 11±2 μM
WiDr: 4±1 μM



MIAPaCa-2: 14±3 μM
MDA-MB-231: 13±2 μM
4T1: 12±3 μM
WiDr: 4±1 μM

**Figure 2.4:** $IC_{50}$ values for carboxycarbonyl allyl esters **15** and **16**

Encouraged by significant cell proliferation inhibition at low micromolar concentrations, we planned to synthesize and explore the biological properties of several carboxy containing NSAIDs. In this regard, we chose six other commercially available NSAIDs, namely ketoprofen **17**, ibuprofen **18**, fenamic acid **19**, mefenamic acid **20**, meclofenamic acid **21** and niflumic acid **22** (**Figure 2.5**). Ibuprofen is an over-the-counter NSAID used for pain, ketoprofen is typically used for toothaches. Fenamic acid itself is not an NSAID but it is the parent molecule for the three NSAIDs mefenamic acid, meclofenamic acid, and niflumic acid. All three of these are commonly used for joint or muscle pain as well as pains related to menstruation such as cramps or headaches. All these NSAIDs provide the therapeutic benefit by inhibition of COX-1 and COX-2.

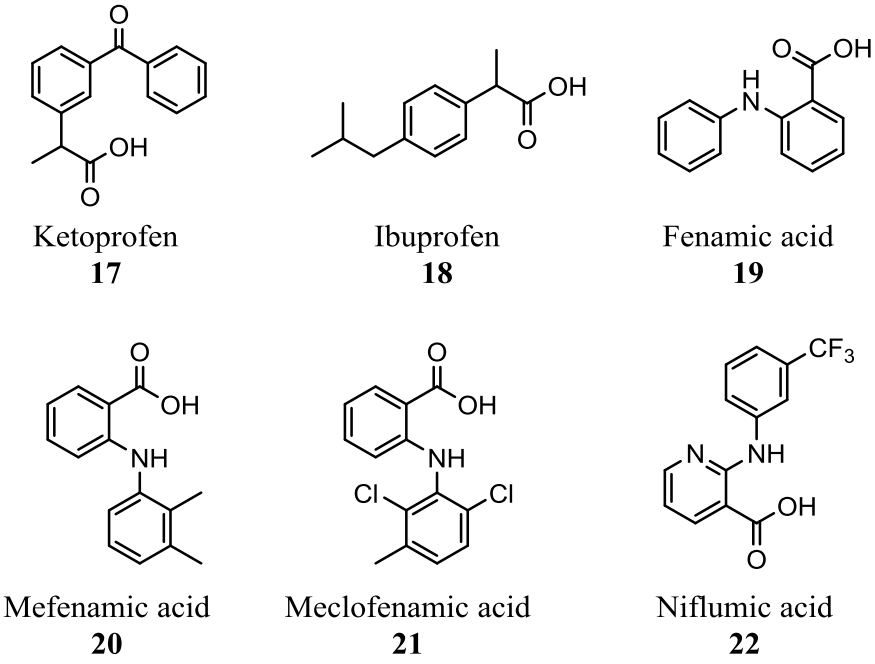
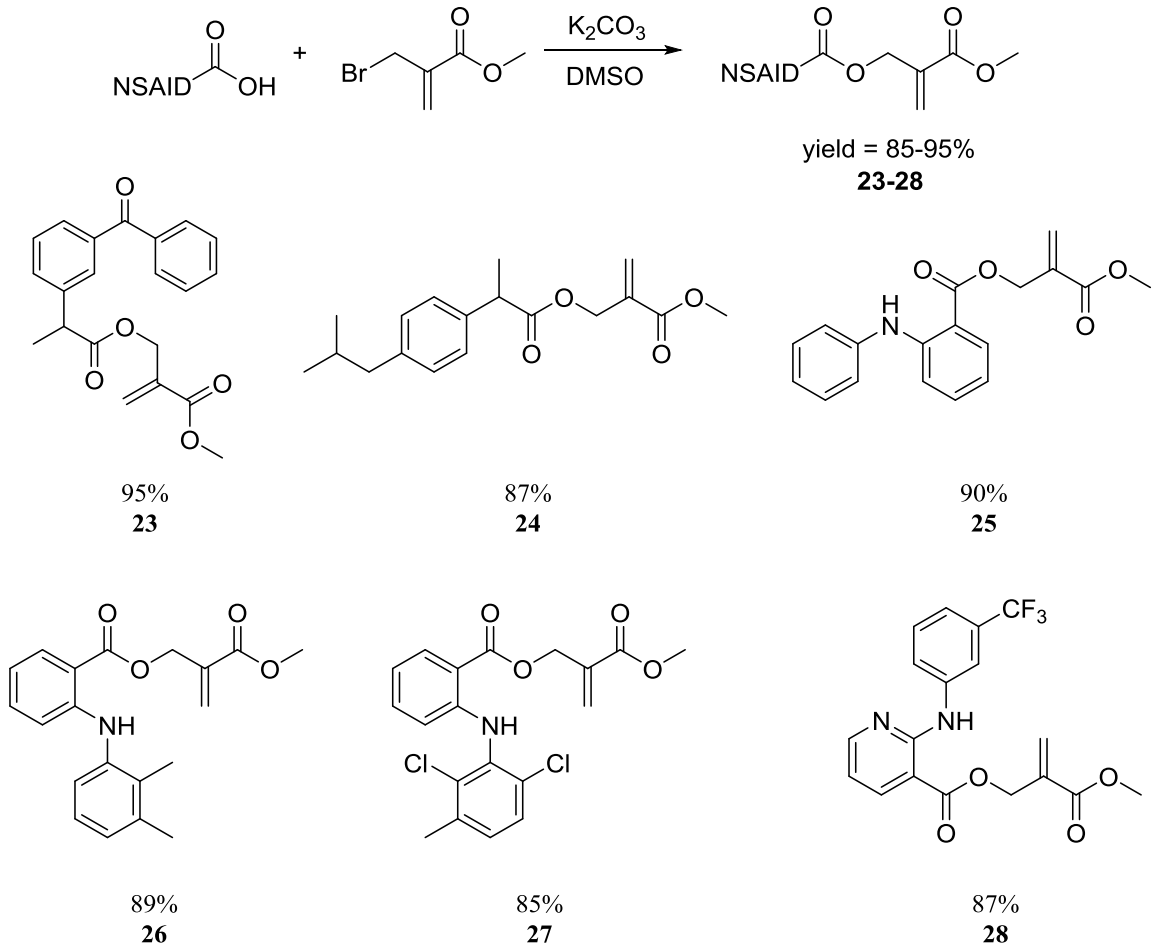



**Figure 2.5:** Some of the carboxylic acid containing NSAIDs

Synthesis of carboxycarbonyl allyl esters **23** to **28** involved the similar procedure employed in scheme **2.4**, by stirring the NSAID carboxylic acids with BH bromide **1** in the presence of potassium carbonate in DMSO at room temperature (**Scheme 2.5**). All the crude products were purified by silica gel column chromatography with a mixture of hexanes and ethyl acetate as eluents. All the pure products were characterized by $^{1}H$ NMR, $^{13}C$ NMR, and mass spectroscopy.

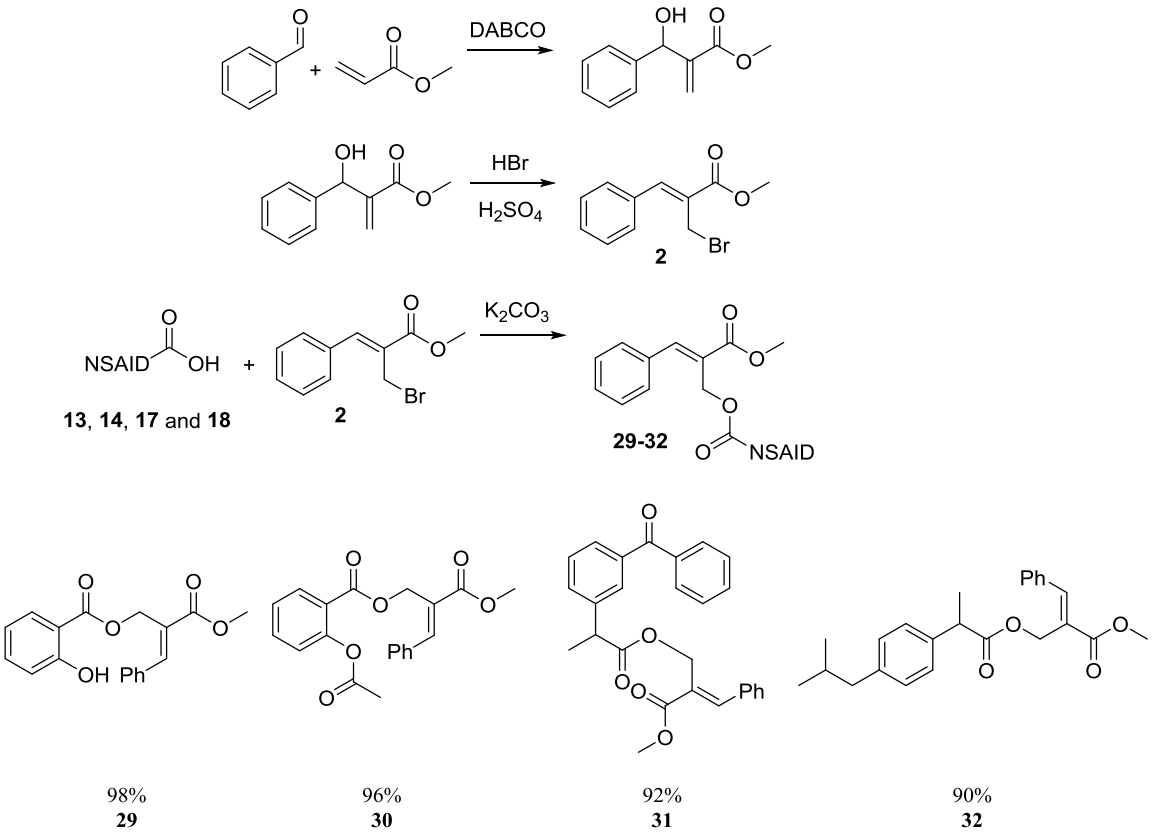



**Scheme 2.5:** Synthesis and $IC_{50}$ values for carboxycarbonyl allyl esters **23-28**

The cell proliferation inhibition studies were carried out using MTT assay as described above. The BH esters derived from aliphatic carboxylic acid NSAIDs ketoprofen and ibuprofen **23**, **24** showed modest cytotoxic properties against all four cancer cell lines. Carboxycarbonyl allyl ester of ketoprofen **23** provided $IC_{50}$ values in the range from 17 µM to >100 µM. Carboxycarbonyl allyl ester of ibuprofen **24** exhibited $IC_{50}$ values in the range from 80 µM to >100 µM. These modest cell proliferation inhibition properties are consistent with our earlier published work that aliphatic carboxylic acids in general provide less cytotoxic properties than aromatic carboxylic acids.[29] The other aromatic carboxylic acid containing NSAID

carboxycarbonyl allyl esters **25-28** showed significant cell proliferation inhibition against all four cell lines. Carboxycarbonyl allyl esters of fenamic acid **25**, mefenamic acid **26**, meclofenamic acid **27** and niflumic acid **28** exhibited $IC_{50}$ values ranged from 16-37 µM, 5-21 µM, 5-21 µM, and 15-25 µM, respectively.

Encouraged by significant increase in cytotoxic values of carboxycarbonyl allyl esters **23-28** derived from formaldehyde BH bromide **1**, we also synthesized carboxycarbonyl allyl esters **29-32** using benzaldehyde BH bromide **2** and NSAIDs **13**, **14**, **17** and **18**. The corresponding BH bromide **2** was synthesized in two steps starting from benzaldehyde which upon condensation with methyl acrylate in the presence of DABCO provided the BH alcohol, which was then brominated using HBr and $H_2SO_4$. The bromide **2** upon reaction with NSAIDs **13**, **14**, **17** and **18** in the presence of potassium carbonate in DMSO provided the crude esters **29-32** (**Scheme 2.6**). The pure products were readily obtained using silica gel chromatography using a mixture of hexane and ethyl acetate as eluents. All the synthesized products were characterized by $^{1}H$ NMR, $^{13}C$, and mass spectrometry.

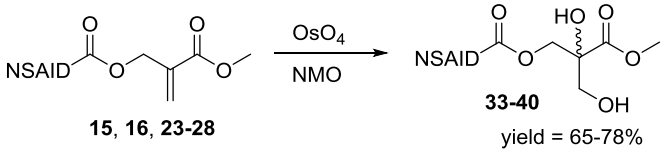



**Scheme 2.6:** Synthesis of benzaldehyde BH ester **2** and the corresponding NSAID esters **29-32**

MTT based cell proliferation inhibition properties against two breast cancer cell lines, MDA-MB-231 and 4T1 of carboxycarbonyl esters **29-32** exhibited slightly decreased $IC_{50}$ values compared to carboxycarbonyl allyl esters **23-28** derived from formaldehyde BH bromide **1**. Again, the aromatic carboxycarbonyl allyl esters of NSAIDs salicylic acid **29** and acetylsalicylic acid **30** provided higher cell proliferation inhibition than aliphatic carboxycarbonyl allyl esters of ketoprofen **31** and ibuprofen **32**. Owing to the decreased cell proliferation inhibition properties and lower water solubility, we did not synthesize carboxycarbonyl allyl esters from the NSAIDs **19-22**. From this SAR study, it is quite evident that β-unsubstitution is more preferred than β-

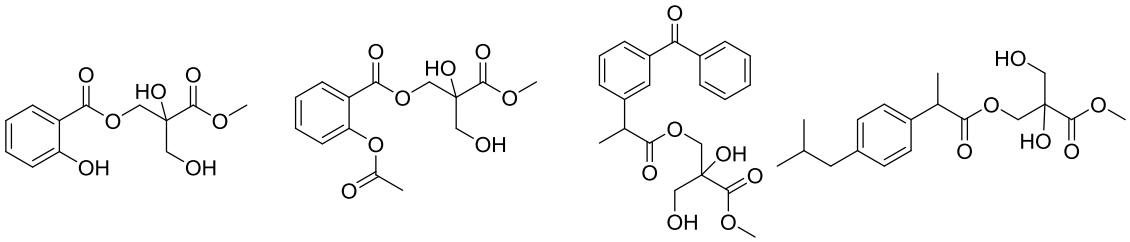
substitution in the double bond region. These results also provide credence for our hypothesis that $S_N2$' mechanism may be operative in providing the biological activity.

Since our proposed mechanism for enhanced cell proliferation property involves the double bond for $S_N2$, $S_N2$' and 1,4 addition, we sought to understand its critical role by saturating the double bond. For this purpose, we chose dihydroxylation of double bond in carboxycarbonylallyl esters **15**, **16**, **23-28**. These allyl esters were treated with a catalytic amount of osmium tetroxide in the presence of N-methylmorphiline N-oxide in acetone to afford the crude diols **33-40** (**Scheme 2.7**). The crude compounds were purified by silica gel column chromatography by using hexane and ethyl acetate (1:1) as eluents to obtain pure products in 65 - 78% yield.




**15**, **16**, **23-28** **33-40**

yield = 65-78%

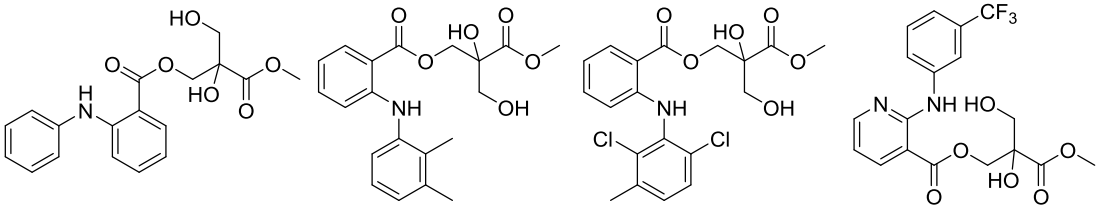



71% **33**

68% **34**

68% **35**

72% **36**




74% **37**

78% **38**

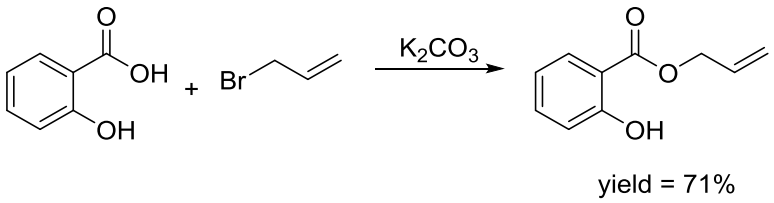
68% **39**

65% **40**

**Scheme 2.7:** General scheme for dihydroxylation of carboxycarbonyl allyl esters

Cell proliferation studies using MTT assay indicated that none of these diolic carboxycarbonyl esters **33-40** did not exhibit any significant inhibition even at 100 µM concentration against all four cancer cell lines. These results are not surprising as we have observed similar loss of activity in the case of simple carboxylic acids in our earlier work.[29]

As a part of SAR study, we synthesized a simple allyl ester **41** from salicylic acid **13** which has limited capability of $S_N2$ or $S_N2'$. Reaction of salicylic acid with allyl bromide under basic conditions provided the corresponding allyl ester **41** in 71% yield (**Scheme 2.8**). As expected, the biological evaluation of **41** against two breast cancer cell lines, MDA-MB-231 and 4T1 indicated no cell proliferation inhibition properties even at 100 µM concentration. The loss of cytotoxic activity also reaffirms the critical role of the double bond with α-carboxycarbonyl group in providing the biological activity via $S_N2$, $S_N2'$, and 1,4 addition pathways.




**Scheme 2.8:** Synthesis of salicylic acid allyl ester **41**

## Conclusion and Future Directions

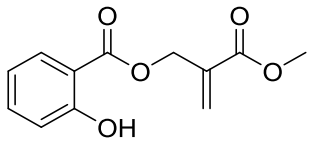
In conclusion, we have synthesized, purified, and characterized several new compounds based on various commercially available NSAIDs. All the synthesized compounds were evaluated for cell proliferation inhibition using MTT assay against four cancer cell lines, MDA-MB-231, 4T1, MiaPaCa-2, and WiDr. All the 2-alkoxycarbonyl allyl esters conjugates of NSAIDs exhibited significantly higher cell proliferation inhibition than the parent NSAIDs. In general, β-unsubstitution in the double bond provided higher biological activity than β-substituted derivatives. Aromatic carboxylic acid containing NSAID esters of BH bromides exhibited higher cell proliferation inhibition than aliphatic carboxylic acid containing NSAIDs. Structure activity relationship studies also indicated that double bond in 2-alkoxycarbonyl allyl ester was a critical component for cell proliferation inhibition, and saturation of the double bond resulted in complete loss of activity, confirming that $S_N2/S_N2'$ mechanism plays a key role in providing the biological activity. Based on the higher cell proliferation inhibition properties, four carboxycarbonylallyl esters derived from salicylic acid, acetyl salicylic acid, mefenamic acid and meclofenamic acid were designated as lead candidate compounds for further studies.

The future directions of this project will involve evaluation of COX inhibition of all the lead compounds and identification of primary lead candidate compound that exhibits higher cell proliferation inhibition, and COX inhibition. The primary lead will also be evaluated for systemic toxicity study in healthy CD-1 mice and anticancer efficacy studies in mice tumor models using WiDr colorectal adenocarcinoma cell line and pancreatic cancer cell line MiaPaCa-2.

## CHAPTER 3: Experimental Procedures and Spectral Characterization

### General procedure for the synthesis of 2-(bromomethyl)acrylate-NSAIDs

A mixture of NSAID (1.0 eq, 42.7 mmol) and methyl 2-(bromomethyl)acrylate (1.1 eq, 46.9 mmol) in 30ml DMSO were first stirred at room temperature for 30 minutes, followed by the addition of potassium carbonate (3.0 eq, 128 mmol). The reaction mixture was stirred for 1 hour at rt. After it was confirmed that the reaction was completed using a TLC (10% EtOAc/hexane), water was added to the reaction mixture in order to dissolve the excess potassium carbonate, and extracted with three times diethyl ether. The crude product was purified by silica gel column chromatography using hexanes and ethyl acetate as eluents.



2-(methoxycarbonyl)allyl 2-hydroxybenzoate

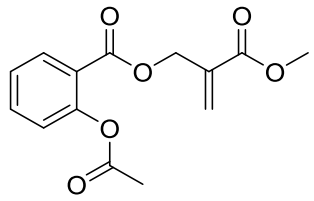
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 10.61 (s, 1H), 7.83 (dd, *J* = 2.0, 4.5 Hz, 1H), 7.43 (m, 1H), 6.94 (dd, *J* =1, 8.5 Hz, 1H), 6.84 (m, 1H), 6.41 (s, 1H), 5.93 (s, 1H), 5.05 (s, 2H), 3.78 (s, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 169.40, 165.38, 161.73, 135.89, 134.77, 129.83, 127.98, 119.20, 117.61, 112.12, 62.99, 52.09

**HRMS (ESI) m/z:** calc'd for $C_{12}H_{12}O_5$ $[M+Na]^+$ : 259.0577, found 259.0777



2-(methoxycarbonyl)allyl 2-acetoxybenzoate

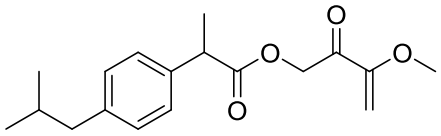
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 8.04 (dd, *J* =1.5, 8 Hz, 1H), 7.57 (m, 1H), 7.32 (m, 1H), 7.11 (dd, *J* = 1.5, 8.5Mz, 1H), 6.42 (s, 1H), 5.93 (s, 1H), 5.02 (s, 2H), 3.80 (s, 3H), 2.32 (s, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 169.64, 165.59, 163.81, 150.78, 135.03, 134.08, 131.81, 127.9, 126.06, 123.89, 122.96, 63.00, 52.09, 20.98

**HRMS (ESI) m/z:** calc'd for $C_{14}H_{14}O_6$ $[M+Na]^+$ : 301.0683, found 301.0906



3-methoxy-2-oxobut-3-en-1-yl 2-(4-isobutylphenyl)propanoate

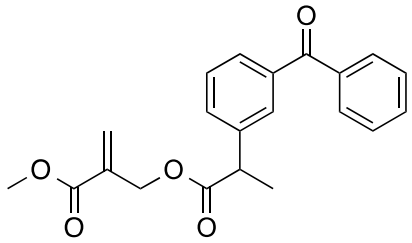
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 7.21 (d, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 6.22 (s, 1H), 5.54 (s, 1H), 4.81 (dd, *J* = 14.0, 50 Hz, 2H), 3.76 (q, *J* = 7.0 Hz, 2H), 3.71 (s, 3H), 2.45 (d, *J* = 7.0 Hz, 2H), 1.87-1.82 (m, 1H), 1.51 (d, *J* = 7.0 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 6H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 173.95, 165.54, 140.65, 137.46, 135.18, 129.33, 127.21, 126.76, 62.31, 51.88, 45.07, 45.00, 30.18, 22.33, 18.24

**HRMS (ESI) m/z:** calc'd for $C_{18}H_{24}O_4$ $[M+Na]^+$ : 327.1567, found 327.1789



methyl 2-(((2-(3-benzoylphenyl)propanoyl)oxy)methyl)acrylate

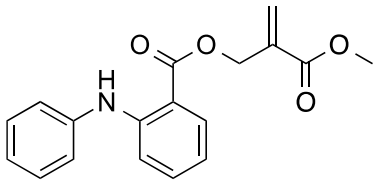
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 7.79-7.4 (m, 8H), 6.26 (s, 1H), 5.64 (s, 1H), 4.80 (dd, *J* = 14.5, 23 Hz, 2H), 3.81 (q, *J* = 7.0 Hz, 1H), 3.70 (s, 3H), 1.55 (d, *J* = 7.0 Hz, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 196.36, 173.32, 165.46, 140.55, 137.95, 137.44, 135.02, 132.51, 131.49, 130.03, 129.20, 129.07, 128.55, 128.31, 127.34, 62.73, 51.97, 45.31, 18.29

**HRMS (ESI) m/z:** calc'd for $C_{21}H_{12}O_5$ $[M+Na]^+$ : 375.1203, found 375.1433



2-(methoxycarbonyl)allyl 2-(phenylamino)benzoate

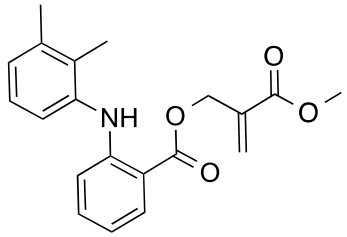
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 9.42 (s, 1H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.36-7.24 (m, 7H), 7.10 (t, *J* = 7.5 Hz, 1H), 6.74 (t, *J* = 7.0 Hz, 1H), 6.43 (s, 1H), 5.97 (s, 1H), 5.06 (s, 2H), 3.82 (s, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 167.72, 165.70, 148.22, 140.62, 135.71, 134.37, 131.60, 129.38, 127.43, 123.70, 122.64, 117.21, 114.05, 111.48, 62.45, 52.11

**HRMS (ESI) m/z:** calc'd for $C18H_{17}NO_4$ $[M+Na]^+$ : 334.105, found 334.127




2-(methoxycarbonyl)allyl 2-((2,3-dimethylphenyl)amino)benzoate

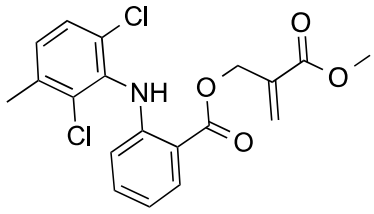
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 9.21 (s, 1H), 8.00 (d, *J* = 10.0 Hz, 1H), 7.26-6.67 (m, 7H), 6.44 (s, 1H), 5.99 (s, 1H), 5.08 (s, 2H), 3.83 (s, 3H), 2.34 (s, 3H), 2.18 (s, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 167.89, 165.73, 149.71, 138.59, 138.23, 135.44, 134.41, 132.54, 131.42, 127.27, 126.88, 125.95, 123.19, 116.08, 113.73, 110.37, 62.31, 52.09, 20.62, 14.00

**HRMS (ESI) m/z:** calc'd for $C_{20}H_{21}NO_2$ $[M+Na]^+$ : 362.1363, found 362.1603




2-(methoxycarbonyl)allyl 2-((2,6-dichloro-3-methylphenyl)amino)benzoate

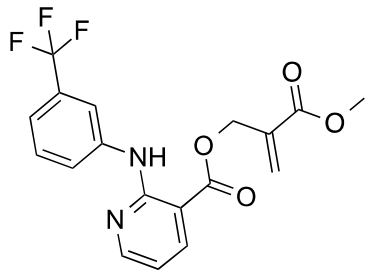
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 9.28 (s, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.32-7.26 (m, 2H), 7.12 (d, *J* = 8.5 Hz, 1H), 6.77 (t, *J* = 8.0 Hz, 1H), 6.45 (s, 1H), 6.33 (d, *J* = 8.5 Hz, 1H), 6.00 (s, 1H), 5.09 (s, 2H), 3.83 (s, 3H), 2.42 (s, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 167.75, 165.73, 147.73, 136.49, 135.33, 135.08, 134.34, 134.22, 131.31, 131.28, 128.59, 127.73, 127.51, 117.37, 113.79, 111.26, 62.51, 52.09, 20.63

**HRMS (ESI) m/z:** calc'd for $C_{19}H_{17}Cl_2NO_4$ $[M+Na]^+$ : 416.0427, found 416.0687



2-(methoxycarbonyl)allyl 2-((3-(trifluoromethyl)phenyl)amino)nicotinate

**$^{1}$H NMR (500 MHz, $CDCl_3$):**

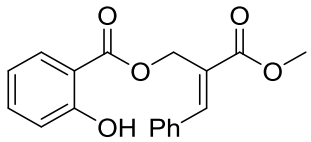
δ 10.28 (s, 1H), 8.42 (d, *J* = 7.0 Hz, 1H), 8.29 (d, *J* = 10.0 Hz, 1H), 8.09 (s, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.43 (t, *J* = 16.0 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 6.80 (m, 1H), 6.46 (s, 1H), 5.97 (s, 1H), 5.09 (s, 2H), 3.83 (s, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 166.79, 165.52, 155.78, 153.32, 140.26, 134.89, 131.12(quartet), 129.18, 128.31, 125.26, 123.55, 123.09, 119.10, 117.16, 114.10, 107.13, 63.23, 52.17

**HRMS (ESI) m/z:** calc'd for $C_{18}H_{15}F_3N_2O_4$ $[M+Na]^+$ : 403.0876 found 403.1146

**Synthesis of Benzaldehyde Baylis Hillman Compounds**




(E)-2-(methoxycarbonyl)-3-phenylallyl 2-hydroxybenzoate

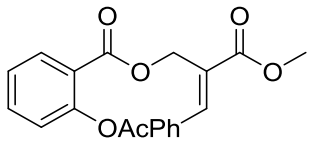
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 10.71 (s, 1H), 8.10 (s, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.37 (m, 6H), 7.00 (d, *J* = 8.5 Hz, 1H), 6.87 (t, *J* = 8.0 Hz, 1H), 5.23 (s, 2H), 3.87 (s, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 169.76, 167.51, 161.68, 146.40, 135.82, 134.06, 130.06, 129.81, 129.43, 128.98, 126.01, 119.21, 117.60, 112.33, 60.09, 52.43

**HRMS (ESI) m/z:** calc'd for $C_{18}H_{16}O_5$ $[M+Na]^+$ : 335.089, found 335.1116




(E)-2-(methoxycarbonyl)-3-phenylallyl 2-acetoxybenzoate

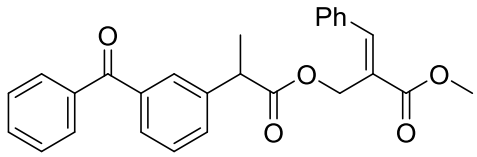
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 8.07 (s, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.57 (m, 1H), 7.42 (m, 5H), 7.32 (t, *J* = 7.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H), 5.16 (s, 2H), 3.86 (s, 3H), 2.29 (s, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 169.61, 167.28, 164.22, 150.75, 146.13, 134.06, 134.01, 132.04, 129.74, 129.52, 128.87, 126.40, 126.07, 123.90, 123.13, 60.07, 52.39, 20.92

**HRMS (ESI) m/z:** calc'd for $C_{20}H_{18}O_6$ $[M+Na]^+$ : 377.0996, found 377.1254




methyl (E)-2-(((2-(3-benzoylphenyl)propanoyl)oxy)methyl)-3-phenylacrylate

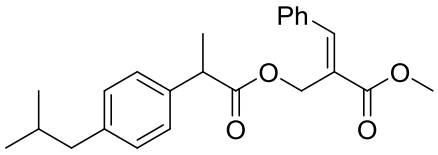
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 7.96 (s, 1H), 7.76-7.20 (m, 14H), 5.00 (d, *J* = 12.0 Hz, 1H), 4.85 (dd, *J* =12, 73 Hz, 1H), 3.87 (q, *J*=7 Hz, 1H), 3.78 (s, 3H), 1.56 (d, *J* = 7.5 Hz, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 196.39, 173.64, 167.81, 145.80, 140.72, 137.98, 137.46, 134.04, 132.49, 131.50, 130.03, 129.58, 129.33, 129.02, 128.65, 128.55, 128.30, 126.35, 59.83, 52.23, 45.34, 18.18

**HRMS (ESI) m/z:** calc'd for $C_{27}H_{24}O_5$ $[M+Na]^+$ : 451.1516, found 451.1825




methyl (E)-2-(((2-(4-isobutylphenyl)propanoyl)oxy)methyl)-3-phenylacrylate

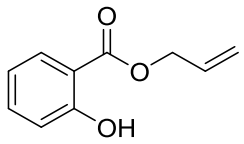
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 7.87 (s, 1H), 7.22-7.02 (m, 9H), 4.80 (dd, *J* = 11.5, 94 Hz, 1H), 3.67 (s, 3H), 2.37 (d, *J* = 7.0 Hz, 2H), 1.76 (m, 1H), 1.43 (d, *J* = 7.0 Hz, 3H), 0.81 (m, 6H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 174.35, 167.31, 145.83, 140.56, 137.65, 134.10, 129.44, 129.39, 128.58, 127.55, 126.52, 59.63, 52.17, 45.07, 45.04, 40.93, 30.20, 22.39, 22.37, 18.27

**HRMS (ESI) m/z:** calc'd for $C_{24}H_{28}O_4$ $[M+Na]^+$ : 403.188, found 403.2148




allyl 2-hydroxybenzoate

**$^{1}$H NMR (500 MHz, $CDCl_3$):**

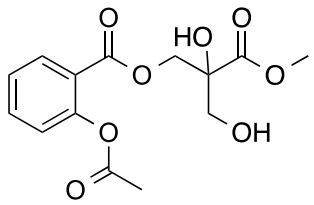
δ 10.71 (s, 1H), 7.85 (s, 1H), 7.42 (s, 1H), 6.95 (s, 1H), 6.85 (s, 1H), 6.00 (m, 1H), 5.40 (d, *J* = 17.0 Hz, 1H), 5.29 (d, *J* = 5.5 Hz), 4.81 (s, 2H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 169.80, 161.72, 135.75, 131.58, 129.92, 119.15, 118.88, 117.59, 112.38, 65.79

**Synthesis of Baylis Hillman NSAID Diols**

To compound (2 mmol) in acetone (10 mL) was added N-methylmorpholine-N-oxide (NMO) (5 mmol) and catalytic osmium tetroxide (0.02 mmol). Reaction was stirred at room temperature for an hour. Upon completion (TLC), acetone was removed under reduced pressure and residue obtained was diluted with 50 mL of water and the crude product was extracted with ethyl acetate (3x25mL) and purified via column chromatography (1:5, EtOAc:Hexanes) to obtain the diol (70%).



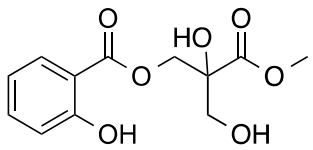

2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl 2-acetoxybenzoate

**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 7.99 (d, *J* = 8.0 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 1H) 7.16 (d, *J* = 8.0 Hz, 1H), 4.50 (s, 2H), 3.93-3.81 (m, 7H), 2.40 (s, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 173.02, 169.85, 163.71, 150.91, 134.29, 131.62, 126.11, 123.89, 122.41, 77.62, 66.13, 64.71, 53.45, 21.00




2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl 2-hydroxybenzoate

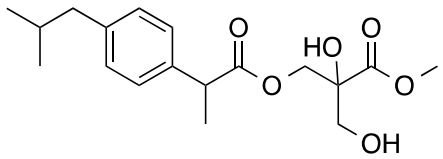
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 10.47 (s, 1H), 7.74 (d, *J* = 10.0 Hz, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 1H), 6.87 (t, *J* = 15.0 Hz, 1H), 4.51 (dd, *J* = 11.5, 21.0 Hz, 2H), 3.92-3.80 (m, 7H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 172.97, 169.40, 161.68, 136.18, 129.82, 119.40, 117.69, 111.77, 110.00, 66.27, 64.68, 53.64

**HRMS (ESI) m/z:** calc'd for $C_{12}H_{14}O_7$ $[M+Na]^+$ : 293.0632, found 293.0848




methyl 2,3-dihydroxy-2-(((2-(4-isobutylphenyl)propanoyl)oxy)methyl)propanoate

**[DIASTEREOMERS] (1:1)**

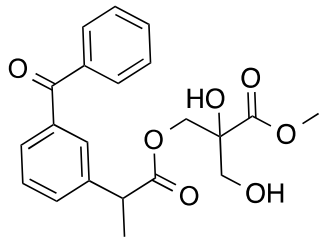
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 7.18-7.08 (m, 8H), 4.26-4.16 (m, 4H), 3.77-3.64 (m, 14H), 2.55-2.43 (m, 6H), 1.88-1.80 (m, 2H), 1.47 (t, *J*=7 Hz, 6H), 0.89 (d, *J* = 7.5 Hz, 12H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 174.19, 172.87, 172.75, 140.75, 140.69, 137.27, 137.11, 129.33, 127.20, 127.17, 66.04, 65.80, 64.62, 64.55, 45.00, 44.98, 44.83, 30.17, 22.33, 18.13, 18.08

**HRMS (ESI) m/z:** calc'd for $C_{18}H_{26}O_6$ $[M+Na]^+$ : 361.1622, found 361.1898




methyl 3-((2-(3-benzoylphenyl)propanoyl)oxy)-2-hydroxy-2-(hydroxymethyl)propanoate

**[DIASTEREOMERS] (1:1)**

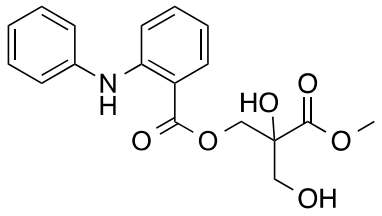
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 7.81 (m, 9H), 4.33-4.21 (m, 2H), 3.85-3.62 (m, 8H), 1.53 (m, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 196.52, 196.48, 173.42, 173.39, 172.78, 172.71, 140.42, 140.33, 138.04, 137.99, 137.36, 132.61, 132.59, 131.52, 131.46, 130.10, 130.09, 129.23, 129.17, 129.11, 128.51, 128.47, 128.33, 66.07, 65.91, 64.59, 64.57, 60.40, 53.32, 53.23, 45.22, 45.19, 21.03, 18.13, 18.11, 14.18

**HRMS (ESI) m/z:** calc'd for $C_{21}H_{22}O_7$ $[M+Na]^+$ : 409.1258, found 409.1557




2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl 2-(phenylamino)benzoate

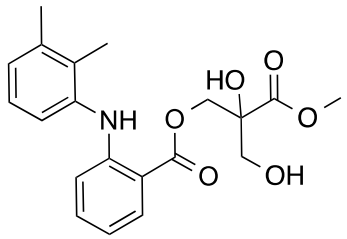
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 9.32 (s, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.36-7.11 (m, 9H), 6.73 (t, *J* = 7.0 Hz, 1H), 4.48 (dd, *J* = 10.5, 32 Hz, 2H), 3.94-3.82 (m, 7H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 173.22, 167.72, 148.30, 140.47, 134.57, 131.54, 129.39, 123.85, 122.78, 117.20, 114.05, 110.96, 65.88, 64.74, 53.55

**HRMS (ESI) m/z:** calc'd for $C_{18}H_{19}NO_6$ $[M+Na]^+$ : 368.1105, found 368.1375




2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl2-((2,3-dimethylphenyl)amino) benzoate

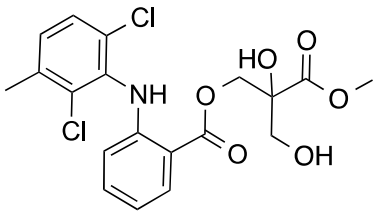
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 9.12 (s, 1H), 7.87 (d, *J* = 8.5 Hz, 1H), 7.27-7.04 (m, 4H), 6.73 (d, *J* = 8.5 Hz, 1H), 6.66 (t, *J* = 8.0 Hz, 1H), 4.49 (dd, *J* = 9.5, 30 Hz, 2H), 3.95-3.82 (m, 7H), 2.33 (s, 3H), 2.17 (s, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 173.26, 167.95, 149.81, 138.45, 138.27, 134.65, 132.64, 131.38, 127.03, 125.99, 123.34, 116.17, 113.71, 109.80, 77.72, 65.87, 64.75, 53.52, 20.61, 13.99

**HRMS (ESI) m/z:** calc'd for $C_{20}H_{23}NO_6$ $[M+Na]^+$ : 396.1418, found 396.1702




2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl2-((2,6-dichloro-3-methylphenyl) amino)benzoate

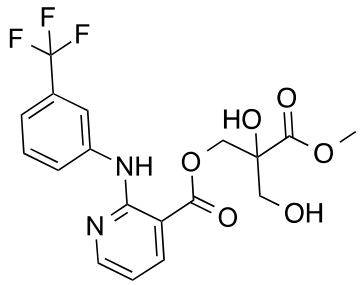
**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 9.18 (s, 1H), 7.90 (d, 1H), 7.32-7.26 (m, 2H), 7.12 (d, J=12.5Hz, 1H), 6.75 (t, *J*= 7.5 1H), 6.31 (d, *J*= 8.5 1H), 4.53 (dd, J=12Hz, J=17 Hz, 1H), 3.86-3.72 (m, 5H), 2.40 (s, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):**

δ 173.26, 167.77, 147.82, 136.52, 134.94, 134.48, 134.36, 131.31, 128.71, 127.75, 117.49, 113.78, 110.71, 77.64, 66.00, 64.75, 53.57, 53.55 20.61

**HRMS (ESI) m/z:** calc'd for $C_{19}H_{19}Cl_2NO6$ $[M+Na]^+$ : 450.0482, found 450.0799




2-hydroxy-2-(hydroxymethyl)-3-methoxy-3-oxopropyl2-((3-(trifluoromethyl)phenyl) amino)nicotinate

**$^{1}$H NMR (500 MHz, $CDCl_3$):**

δ 10.18 (s, 1H), 8.50 (d, *J* = 5.0 Hz, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 8.14 (s, 1H), 7.92 (d, *J* = 8.5 Hz, 1H), 7.50 (t, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 6.87 (m, 1H), 4.59 (dd, *J* = 11.0, 39.5 Hz, 2H), 4.01-3.89 (m, 6H)

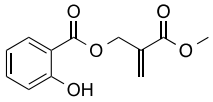
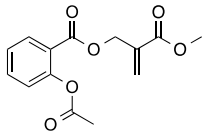
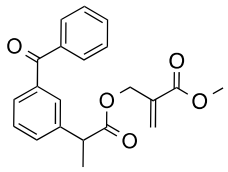
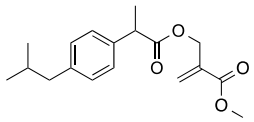
**$^{13}$C NMR (125 MHz, $CDCl_3$):**

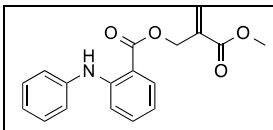
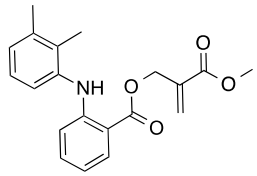
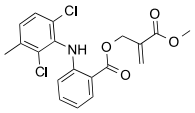
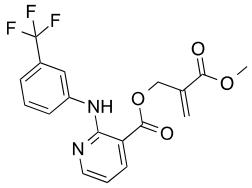
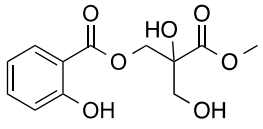
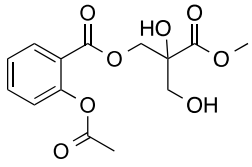
δ 173.06, 166.74, 155.79, 153.58, 140.26, 140.07, 132.04 (quartet), 129.23, 123.73, 119.32, 119.29, 117.37, 114.21, 106.68, 66.30, 64.71, 53.69

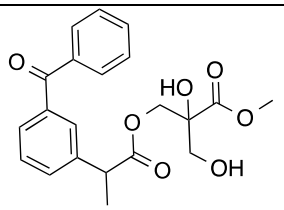
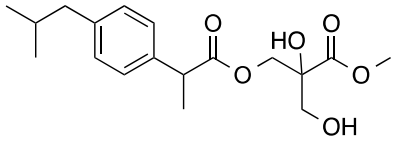
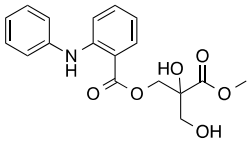
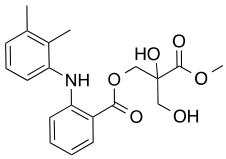
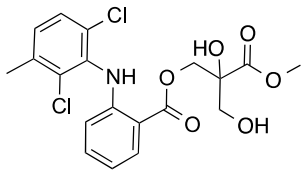
**HRMS (ESI) m/z:** calc'd for $C18H17F3N_2O6$ $[M+H]^+$ : 415.1111, found 415.1395

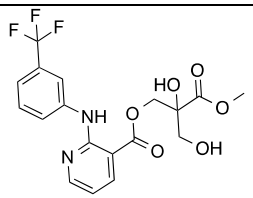
**Biological Evaluation**

**Table 1: $IC_{50}$* values of NSAID-Baylis Hillman derivatives in MiaPaCa-2, MDA-MB-231, 4T1, and WiDr cell lines using MTT assay**

| **Compound** | **MiaPaCa-2** | **MDA-MB-231** | **4T1** | **WiDr** |
|---|---|---|---|---|
| Salicylic Acid Baylis Hillman  | 15±1 | 17±2 | 11±2 | 4±1 |
| Aspirin Baylis Hillman  | 14±3 | 13±2 | 12±3 | 4±1 |
| Ketoprofen Baylis Hillman  | 95±5 | 54±6 | >100 | 17±0 |
| Ibuprofen Baylis Hillman  | >100 | >100 | >100 | 80±5 |
| Fenamic Baylis Hillman | 36±2 | 26±3 | 37±1 | 16±2 |

| | | | | |
|---|---|---|---|---|
|  | | | | |
| Mefenamic Acid Baylis Hillman  | 7±1 | 9±1 | 16±1 | 6±1 |
| Meclofenamic Acid Baylis Hillman  | 7±2 | 10±3 | 21±1 | 5±1 |
| Niflumic Acid Baylis Hillman  | 25±1 | 22±1 | 24±2 | 15±1 |
| Salicyic Acid Baylis Hillman Diol  | >100 | >100 | >100 | >100 |
| Aspirin Baylis Hillman Diol  | >100 | >100 | >100 | >100 |
| Ketoprofen Baylis Hillman | >100 | >100 | >100 | >100 |

| | | | | |
|---|---|---|---|---|
|  Diol | | | | |
| Ibuprofen Baylis Hillman Diol  | >100 | >100 | >100 | >100 |
| Fenamic Acid Baylis Hillman  Diol | >100 | >100 | >100 | >100 |
| Mefenamic Acid Baylis Hillman Diol  | >100 | >100 | >100 | >100 |
| Meclofenamic Acid Baylis Hillman Diol  | >100 | >100 | >100 | >100 |
| Niflumic Acid Baylis Hillman | >100 | >100 | >100 | >100 |

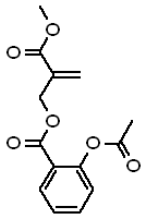
| | | | | |
|---|---|---|---|---|
|  Diol | | | | |

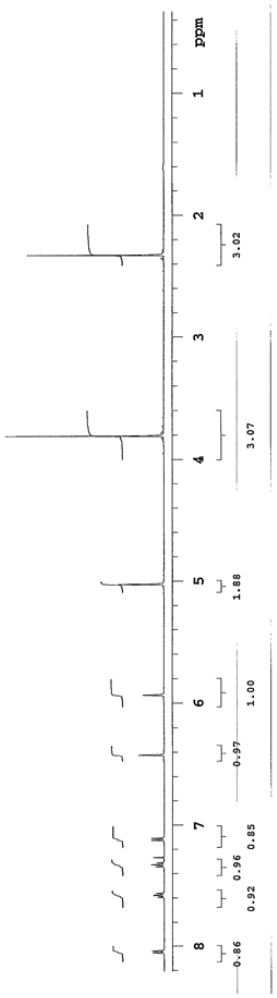
## References

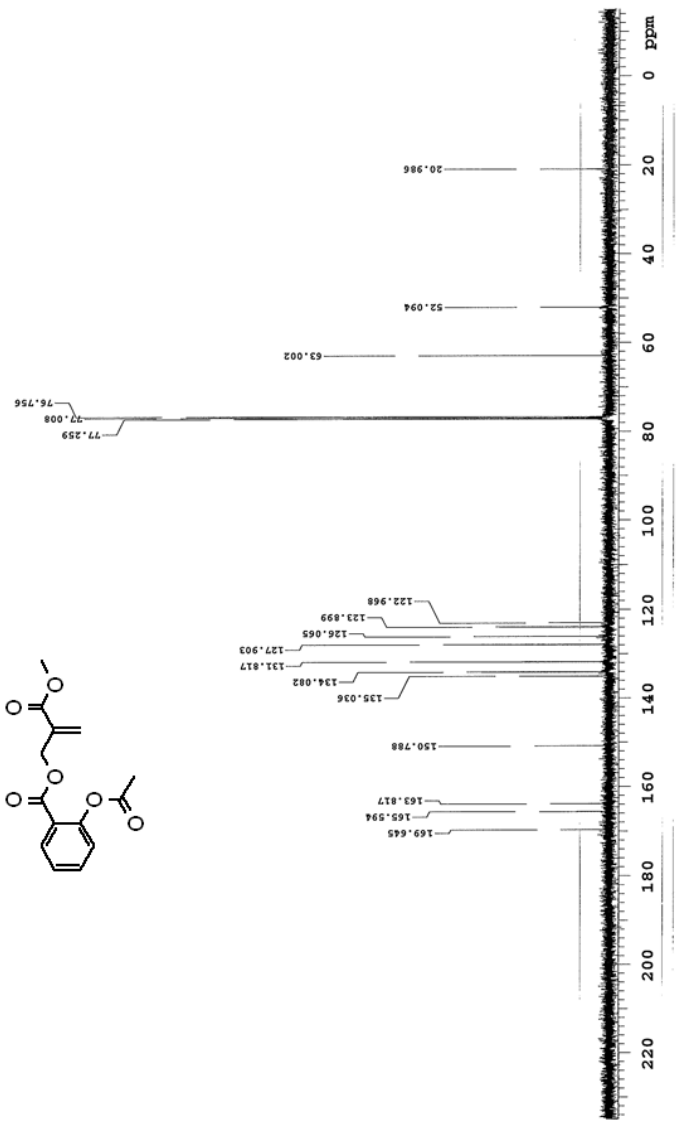

(1) Khosrow K., *Advances in Pharmacology*, 2009, *57*, 31-89.
(2) Marques, J., Gaspar V., Costa E., , Paquete, C., Correia, I., *Biointerfaces*, **2014,** *113*, 375-383
(3) Kunte, D., Wali, R., DeLaCruz, M., Gibson, T., Tiwari, A., Brasky, J., Stypula, Y., Roy, H., *Gastroenterology*, **2011,** *140* (5), 41.
(4) Bézière, N., Goossens, L., Pommery, J., Vezin, H., Touati, Hénichart, JP., Nicole Pommery, N., *Bioorg. Med. Chem. Lett.*, **2008,** *18* (16), 4655-4657.
(5) Hanahan, D.; Weinberg, R. A. *Cell* **2011**, *144* (5), 646–674.
(6) Tabrizi, L.; Chiniforoshan, H.; McArdle, P.; Ebrahimi, M.; Khayamian, T. *Inorganica Chim. Acta* **2015**, *432*, 176–184.
(7) Dokorou, V.; Primikiri, A.; Kovala-Demertzi, D. *J. Inorg. Biochem.* **2011**, *105* (2), 195–201.
(8) Coyle, B.; McCann, M.; Kavanagh, K.; Devereux, M.; McKee, V.; Kayal, N.; Egan, D.; Deegan, C.; Finn, G. J. *J. Inorg. Biochem.* **2004**, *98* (8), 1361–1366.
(9) Santos, R. L. S. R.; Bergamo, A.; Sava, G.; De Oliveira Silva, D. *Polyhedron* **2012**, *42* (1), 175–181.
(10) Rubner, G.; Bensdorf, K.; Wellner, A.; Bergemann, S.; Ott, I.; Gust, R. *Eur. J. Med. Chem.* **2010**, *45* (11), 5157–5163.
(11) Zhang, L.; Li, G.; Gao, M.; Liu, X.; Ji, B.; Hua, R.; Zhou, Y.; Yang, Y. *Colloids Surfaces B Biointerfaces* **2016**, *144*, 81–89.
(12) Vijaya Babu, P.; Ashfaq, M. A.; Shiva Kumar, K.; Mukkanti, K.; Pal, M. *Arab. J. Chem.* **2015**.
(13) Cheng, K. W.; Nie, T.; Ouyang, N.; Alston, N.; Wong, C. C.; Mattheolabakis, G.; Papayannis, I.; Huang, L.; Rigas, B. *Int. J. Pharm.* **2014**, *477* (1–2), 236–243.
(14) Zawidlak-Wegrzyńska, B.; Kawalec, M.; Bosek, I.; Łuczyk-Juzwa, M.; Adamus, G.; Rusin, A.; Filipczak, P.; Głowala-Kosińska, M.; Wolańska, K.; Krawczyk, Z.; Kurcok, P. *Eur. J. Med. Chem.* **2010**, *45* (5), 1833–1842.
(15) Wittine, K.; Benci, K.; Rajić, Z.; Zorc, B.; Kralj, M.; Marjanović, M.; Pavelić, K.; De Clercq, E.; Andrei, G.; Snoeck, R.; Balzarini, J.; Mintas, M. *Eur. J. Med. Chem.* **2009**, *44* (1), 143–151.
(16) Vannini, F.; Mackessack-Leitch, A. C.; Eschbach, E. K.; Chattopadhyay, M.; Kodela, R.; Kashfi, K. *Bioorganic Med. Chem. Lett.* **2015**, *25* (20), 4677–4682.
(17) Jacob, J. N.; Tazawa, M. J. *Bioorg. Med. Chem. Lett.* **2012**, *22* (9), 3168–3171.
(18) Zhang, L.; Liu, L.; Zheng, C.; Wang, Y.; Nie, X.; Shi, D.; Chen, Y.; Wei, G.; Wang, J. *Eur. J. Med. Chem.* **2017**, *131*, 81–91.
(19) Chennamaneni, S.; Zhong, B.; Lama, R.; Su, B. *Eur. J. Med. Chem.* **2012**, *56*, 17–29.
(20) Fogli, S.; Banti, I.; Stefanelli, F.; Picchianti, L.; Digiacomo, M.; Macchia, M.; Breschi, M. C.; Lapucci, A. *Eur. J. Med. Chem.* **2010**, *45* (11), 5100–5107.
(21) Gobec, S.; Brožič, P.; Rižner, T. L. *Bioorganic Med. Chem. Lett.* **2005**, *15* (23), 5170–5175.
(22) Velázquez, C. A.; Praveen Rao, P. N.; Citro, M. L.; Keefer, L. K.; Knaus, E. E. *Bioorganic Med. Chem.* **2007**, *15* (14), 4767–4774.

(23) Lu, X. Y.; Wang, Z. C.; Ren, S. Z.; Shen, F. Q.; Man, R. J.; Zhu, H. L. Bioorganic Med. Chem. Lett. 2016, 26 (15), 3491–3498.
(24) Silva W., Rodrigues, D., Oliveira, R., Mendes, R., Olegário, T., Rocha, J., S. L. Keesen , T., Lima-Junior, C., Vasconcellos, M., Bioorganic Med. Chem. Lett. 2016, 26, 4523-4526
(25) Kunakbaevaa, Z., Carrascoa, R., Rozasb,I., J. Mol. Structure 2003, 626, 209-216
(26) Luciana K. Kohn, L., Pavam, C.H., Veronese, D., Coelho F., De Carvalho J.E., Almeida, W., Eur. J. Med. Chem. 2006, 41, 738-744
(27) Lima–Junior, C., Vasconcellos, M., Bioorganic Med. Chem. Lett. 2012, 20, 3954-3971
(28) Chan, K., O'Brien, P., J. Appl. Toxicol. 2008; 28, 1004-1015
(29) Ronayne, C., Solano, L., Nelson, G., Lueth, E., Hubbard, S., Schumacher, T., Gardner, Z., Jonnalagadda, S., Gurrapu, S., Holy, J., Bioorganic Med. Chem. Lett. 2016, 27 (4), 776-780

**Appendix/Appendices**



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77.008
77.259
122.968
123.899
126.065
127.903
131.817
134.082
135.036
150.788
163.817
165.594
169.645

OH
ppm
11
10
9
8
7
6
5
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3
2
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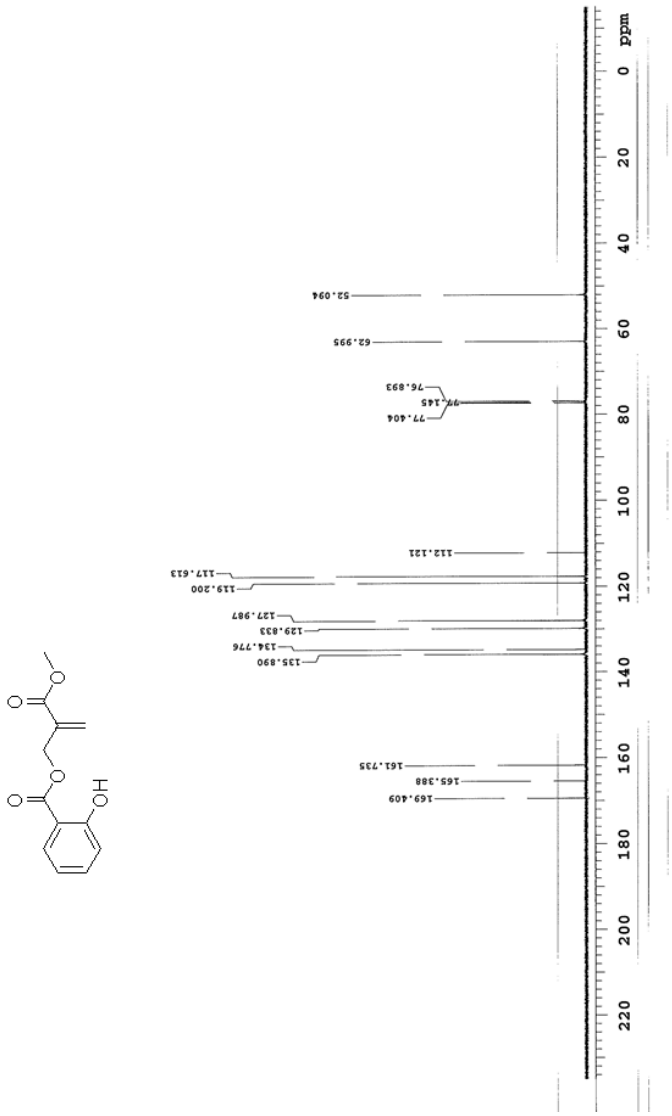
0.87
0.94
0.96
1.94
3.00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112.121
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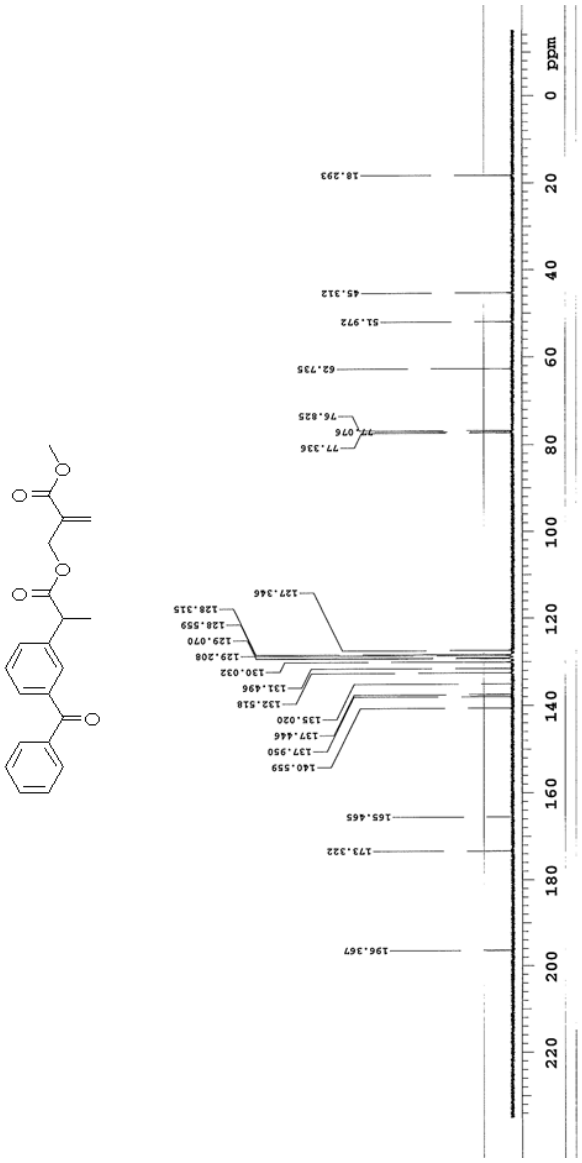
76.893
62.995
52.094
ppm

ppm
1
2
3
4
5
6
7
8
3.22
3.08
1.19
2.08
1.00
0.99
3.15.17
1.113.18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45.312
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62.735
76.835
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128.315
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140.559
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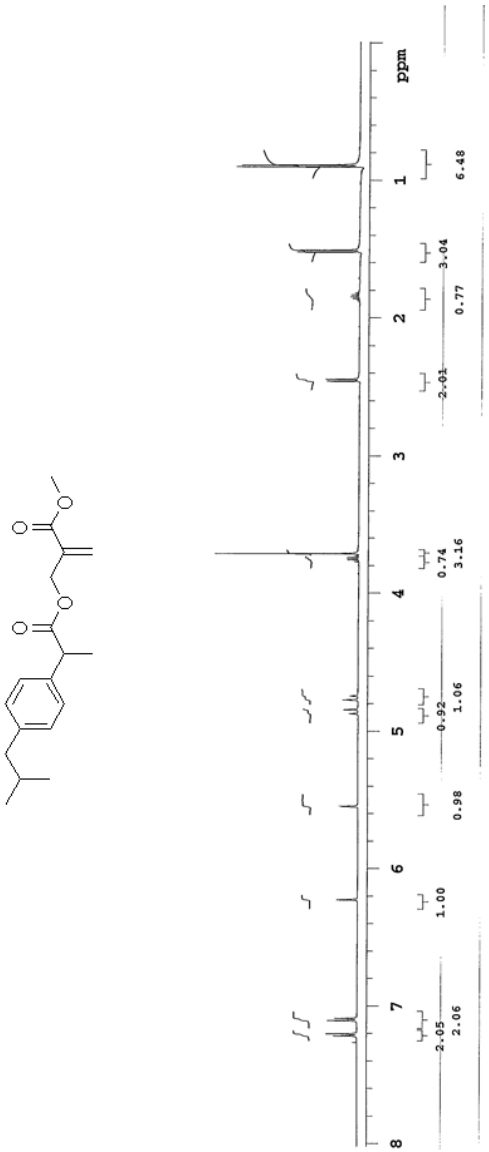
196.367
ppm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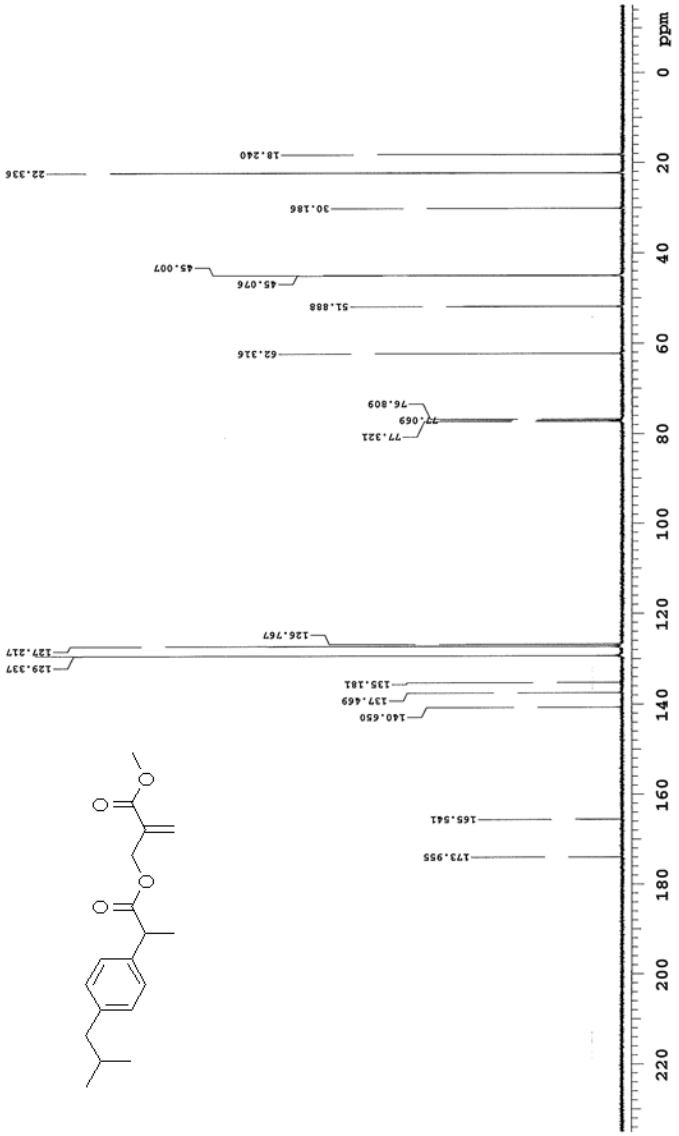
0.98
1.00
2.05
2.06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60
80
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120
140
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180
200
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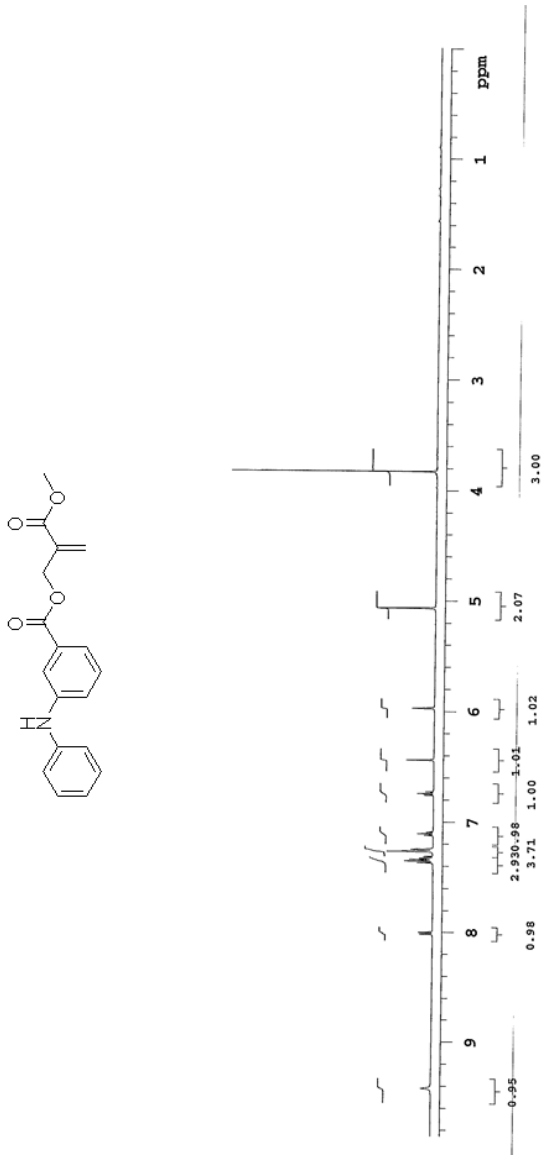
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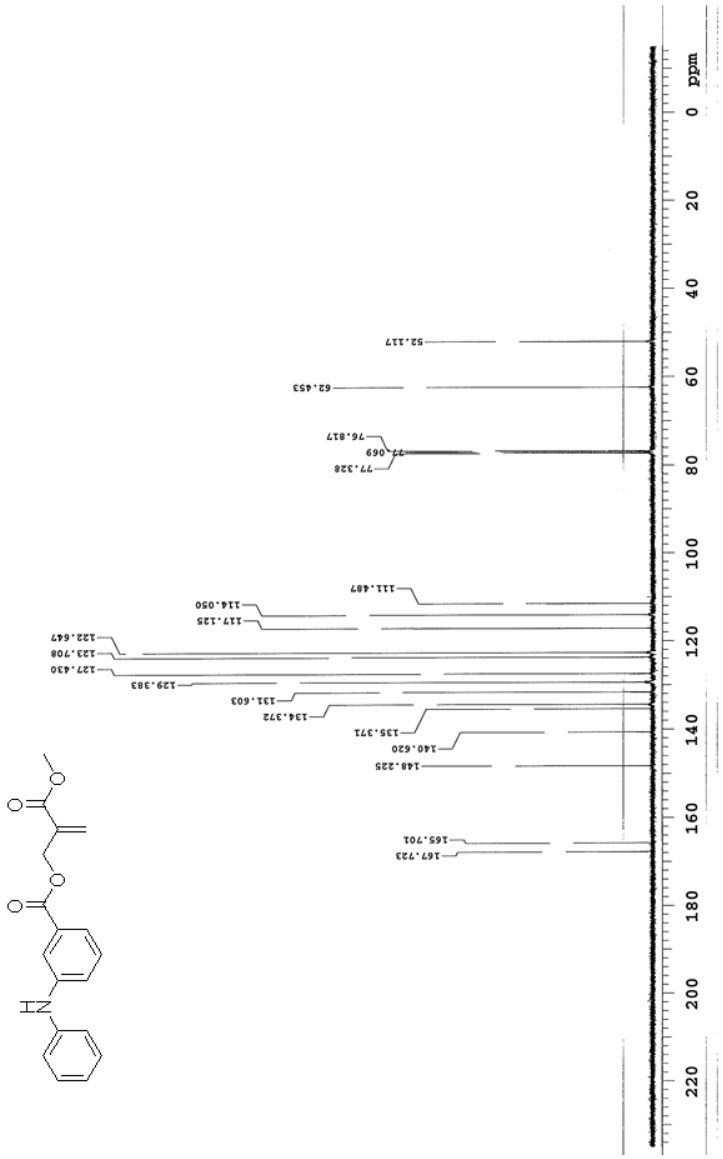
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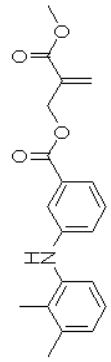
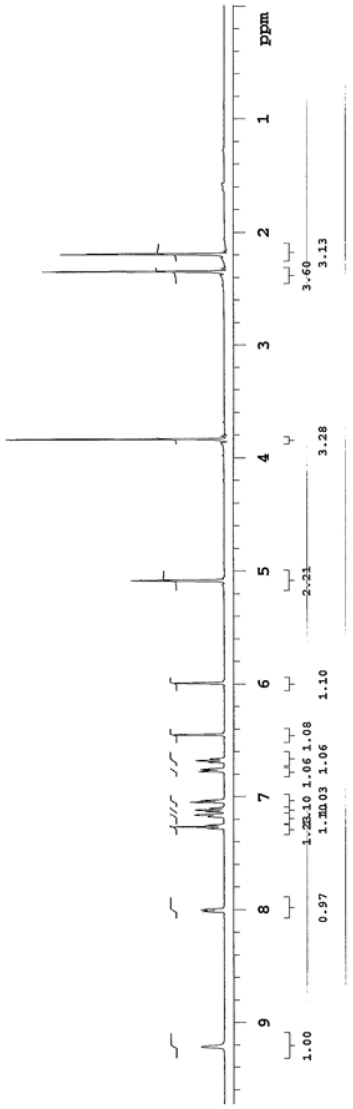
ppm

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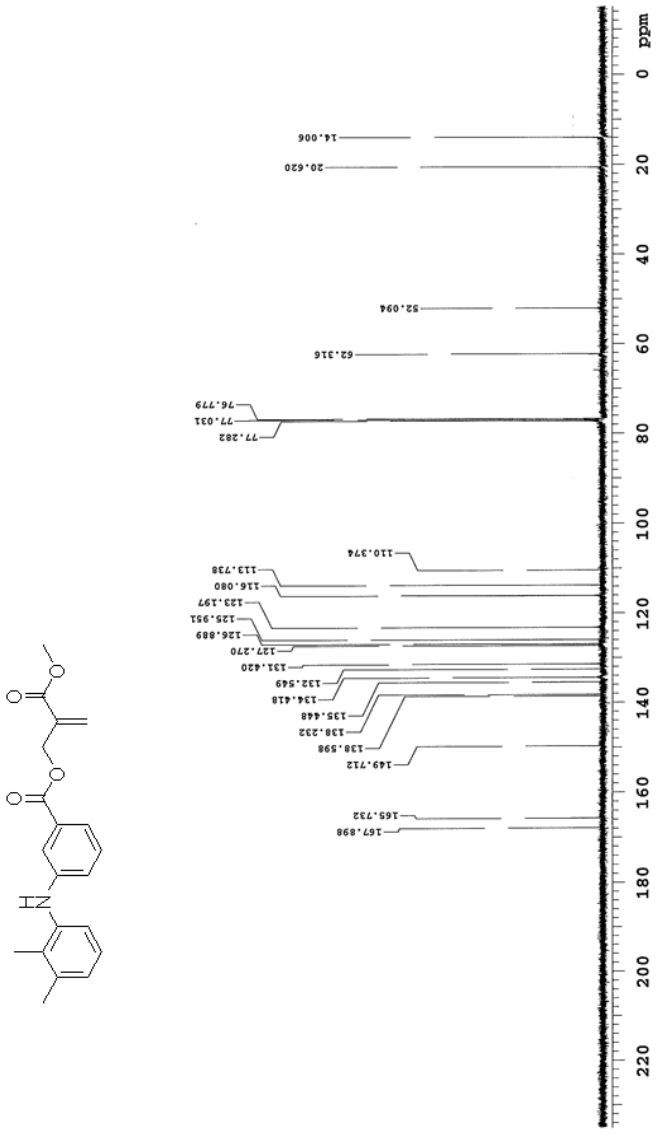
3.13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131.420
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138.598
149.712
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167.898
0
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40
60
80
100
120
140
160
180
200
220
ppm

O
O
O
O
H
N
Cl
Cl


ppm
9
8
7
6
5
4
3
2
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1.01
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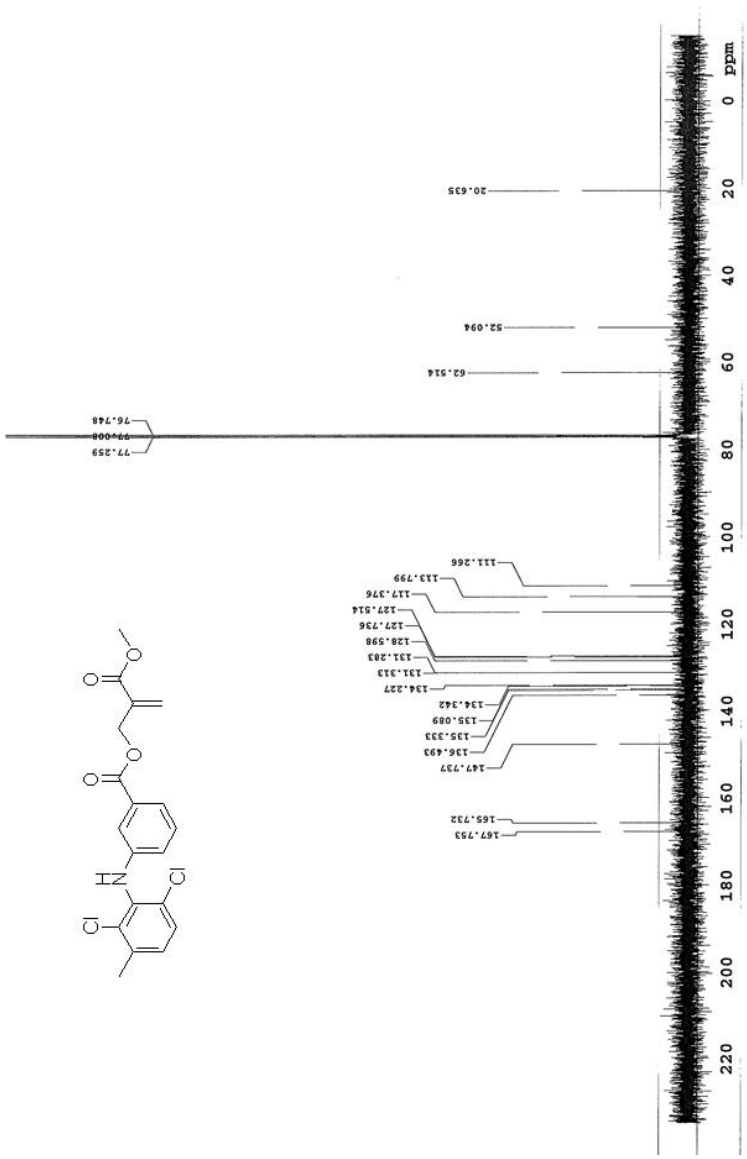
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136.693
147.737
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ppm
0
20
40
60
80
100
120
140
160
180
200
220
O
O
O
O
HN
Cl
Cl

F3C
H
N
N
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O
O
ppm
10
9
8
7
6
5
4
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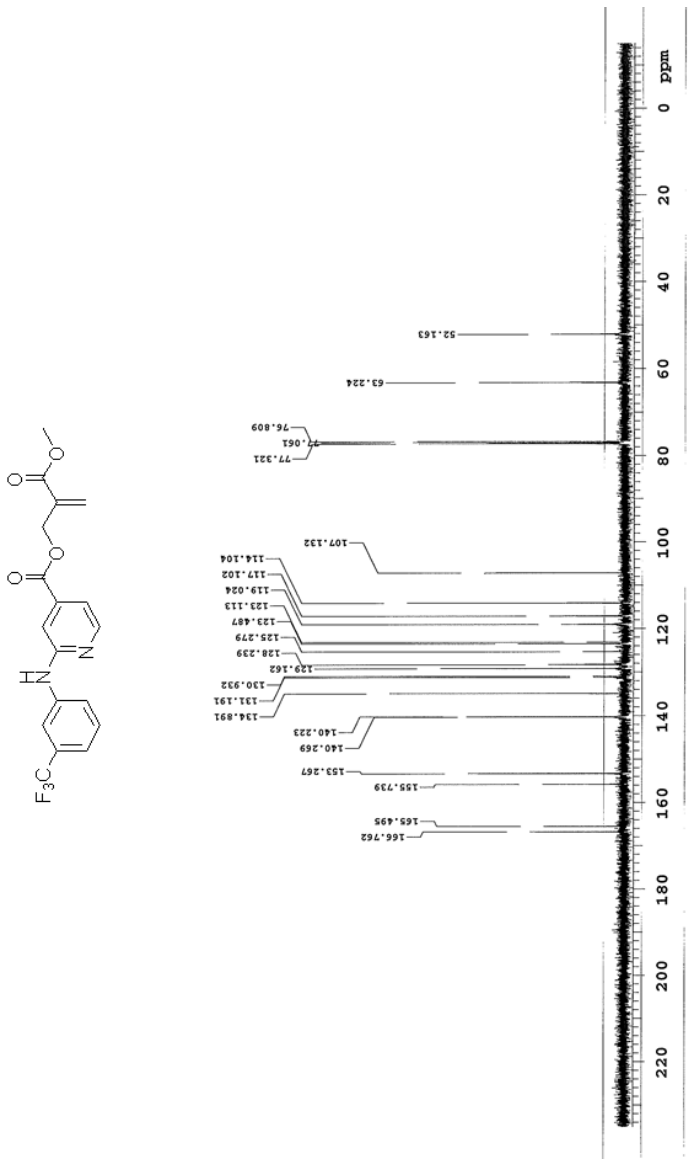
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117.102
119.024
123.113
123.487
125.279
128.239
129.162
130.932
131.191
134.891
140.223
140.269
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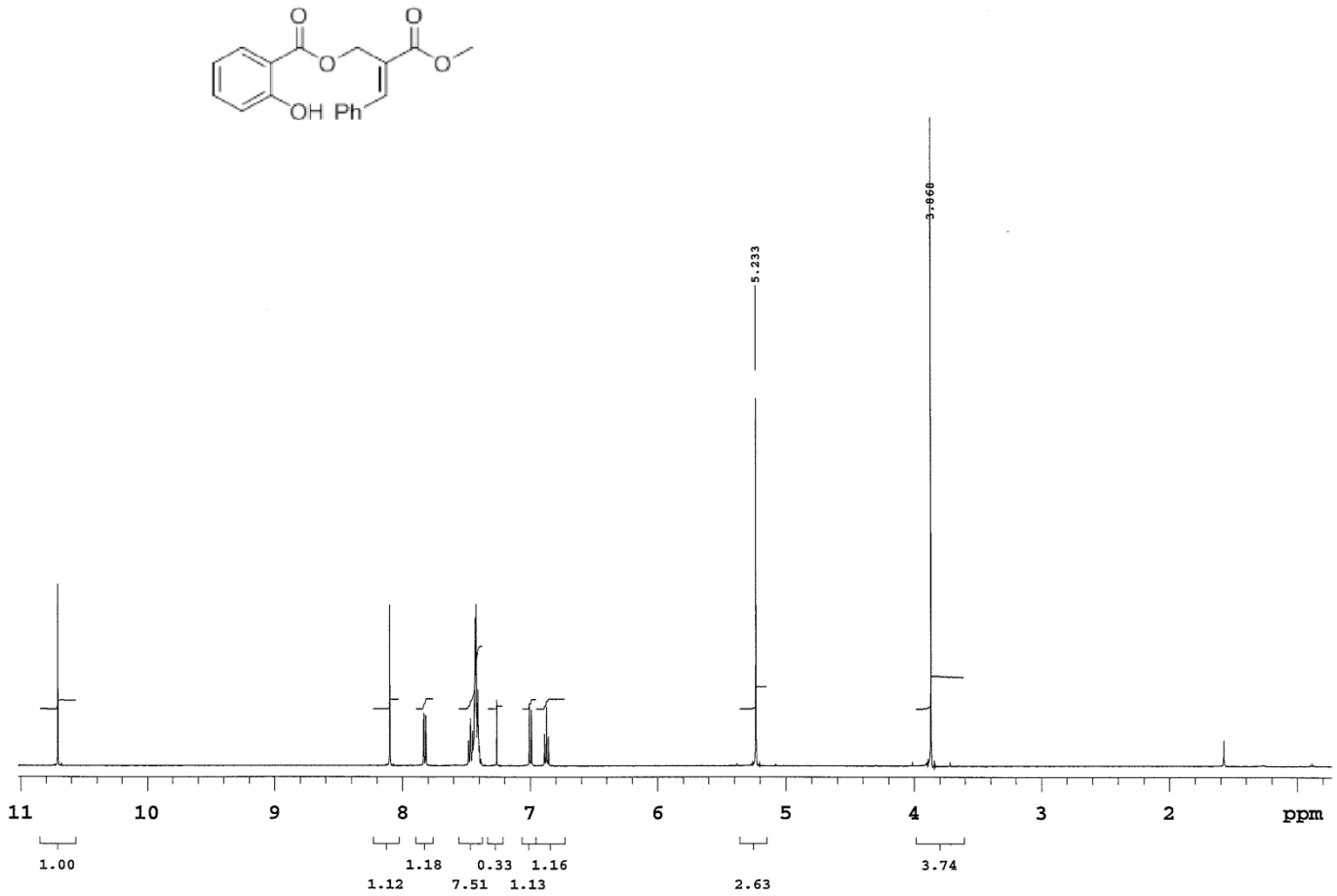
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O
O
O
OH Ph
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3.860
11
10
9
8
7
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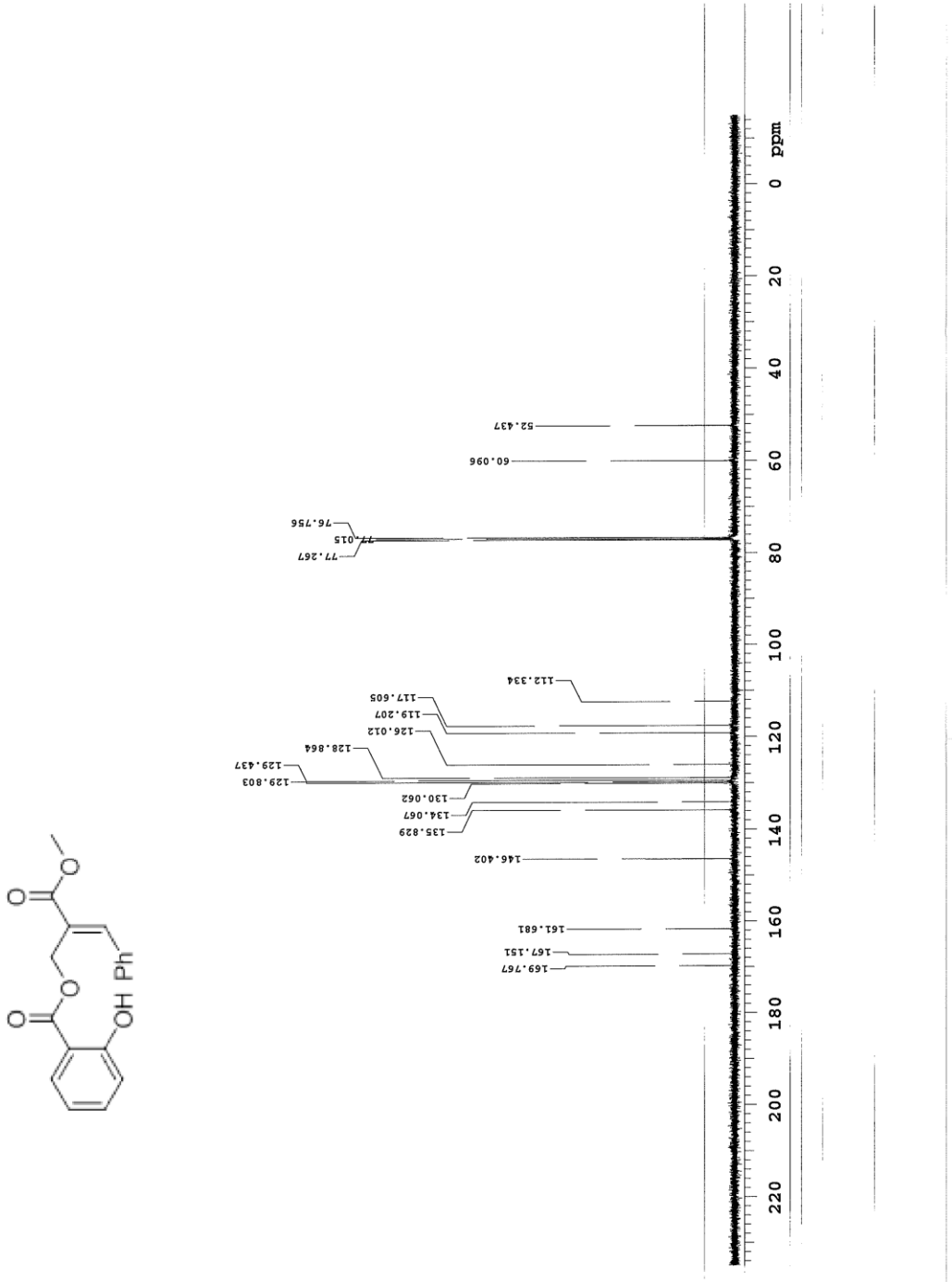
3.74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Ph
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128.864
126.012
119.207
117.605
112.334
77.267
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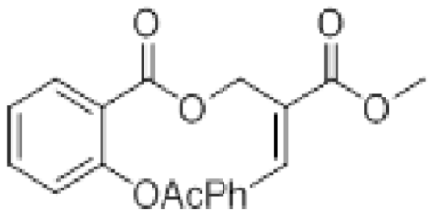
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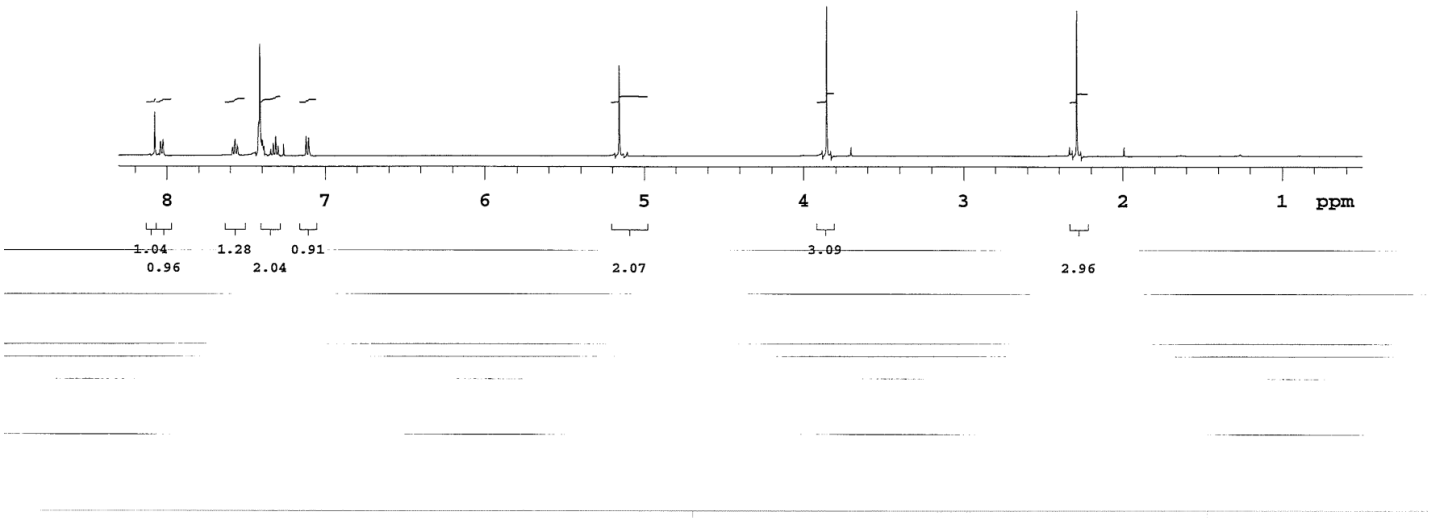
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7
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5
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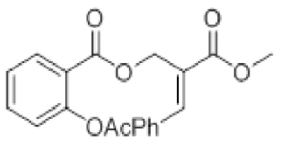
3.09
2.96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O
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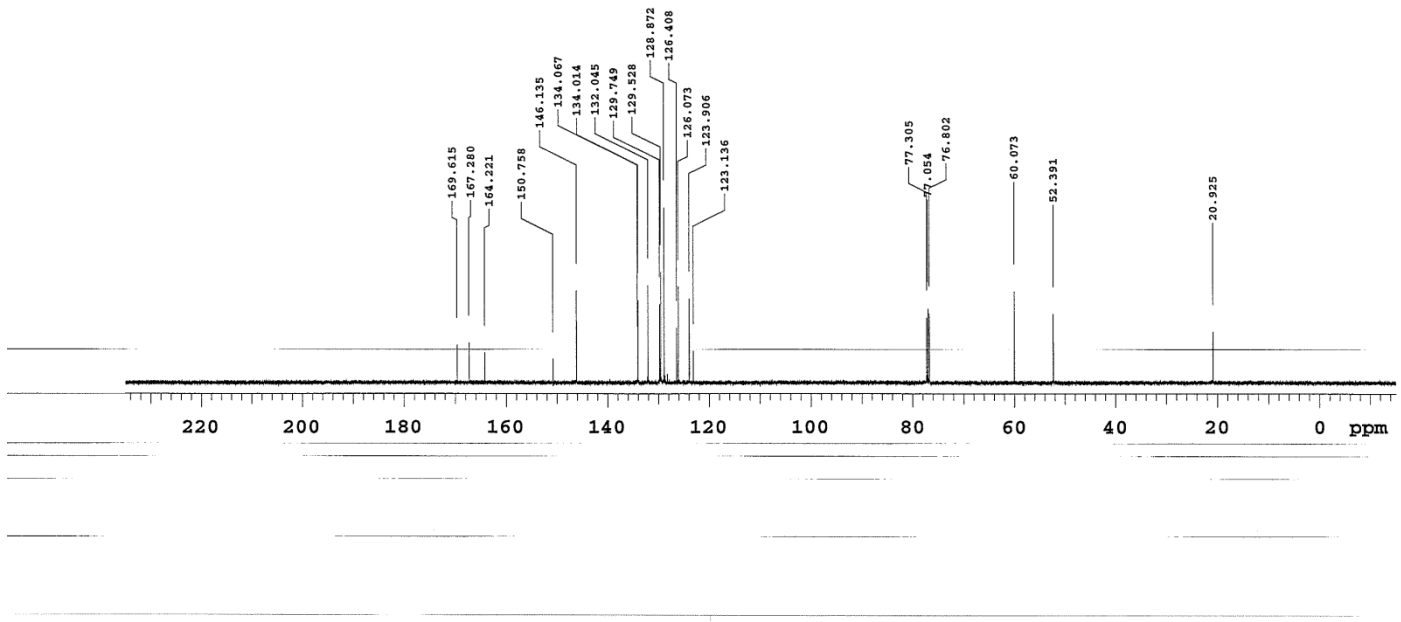
OAcPh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134.014
132.045
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129.528
128.872
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126.073
123.906
123.136
77.305
77.054
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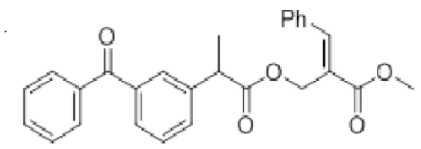
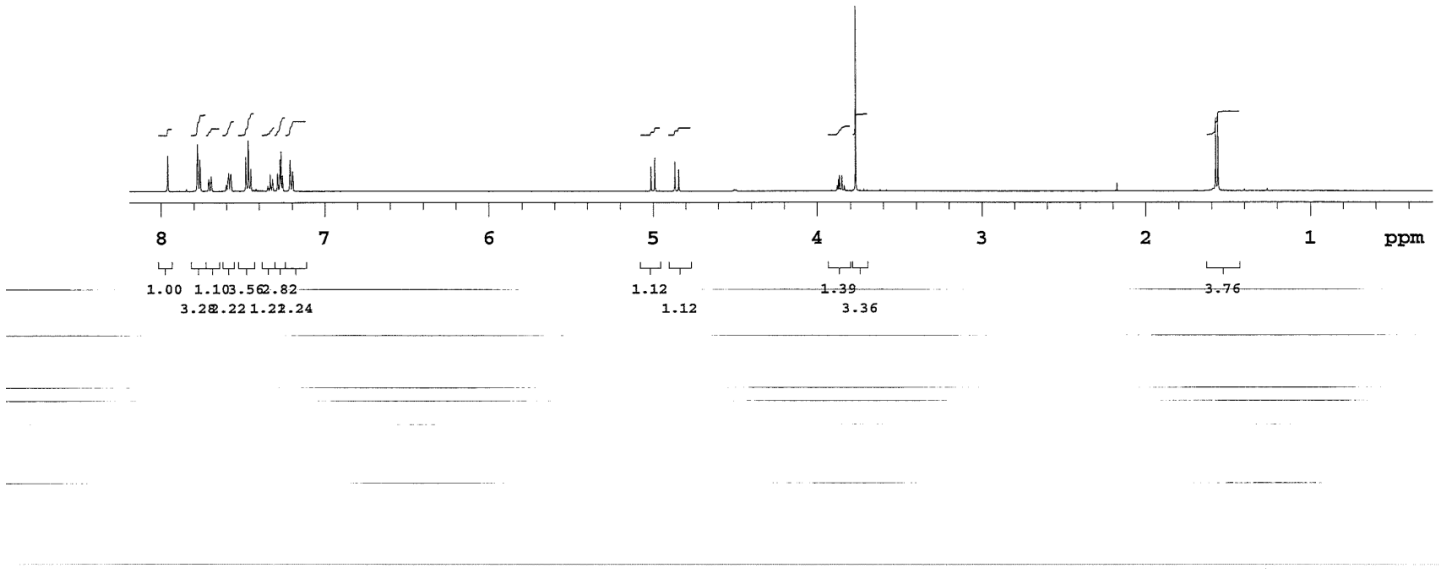
52.391
20.925
220
200
180
160
140
120
100
80
60
40
20
0
ppm


Ph
O
O
O
O
O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7
6
5
4
3
2
1
ppm
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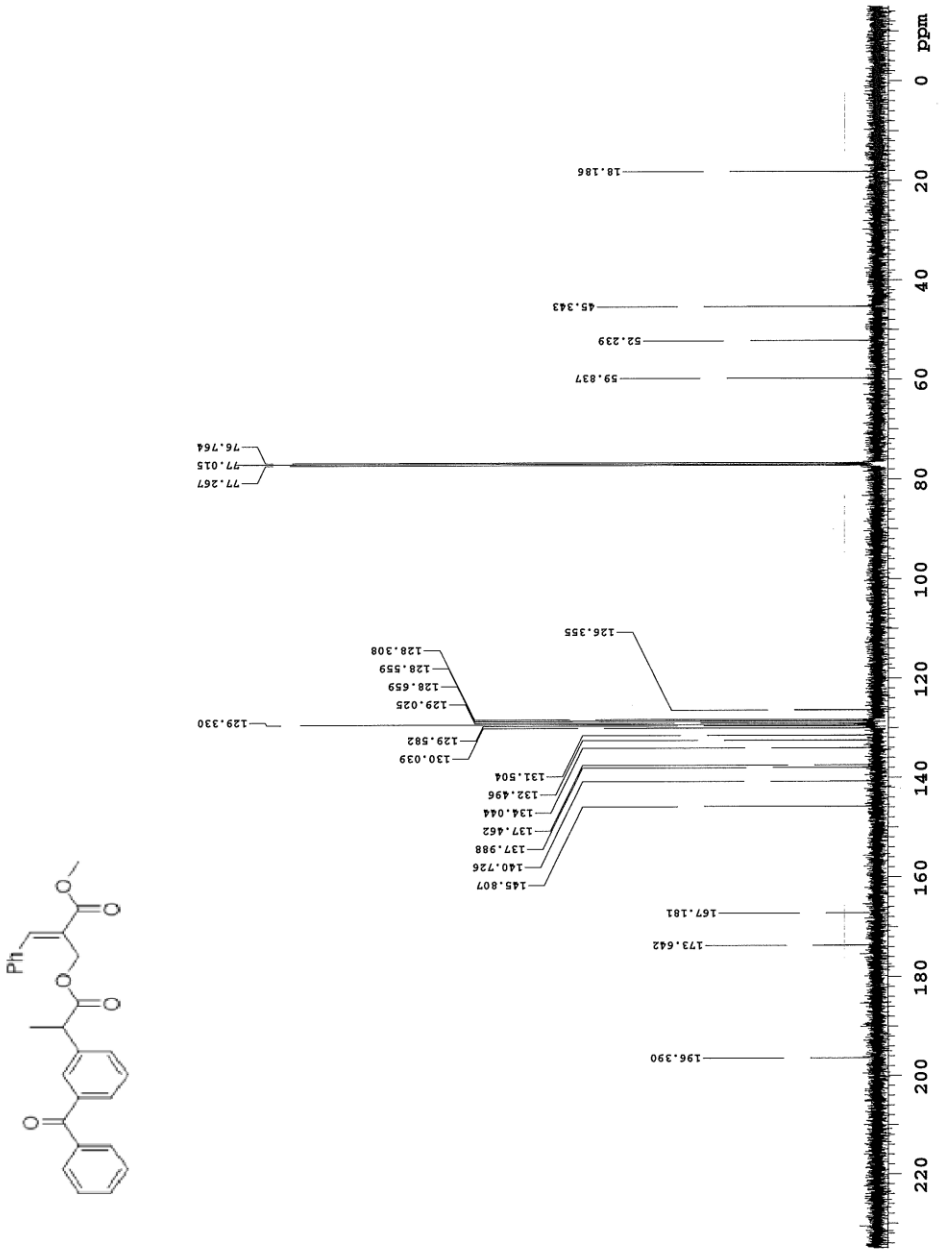
2.22
1.22
2.24
1.12
3.36


Ph
O
O
O
O
O
ppm
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40
60
80
100
120
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160
180
200
220
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45.343
52.239
59.837
76.764
77.015
77.267
126.355
128.308
128.559
128.659
129.025
129.330
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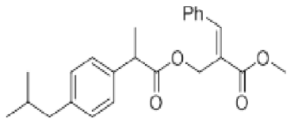
196.390


Ph
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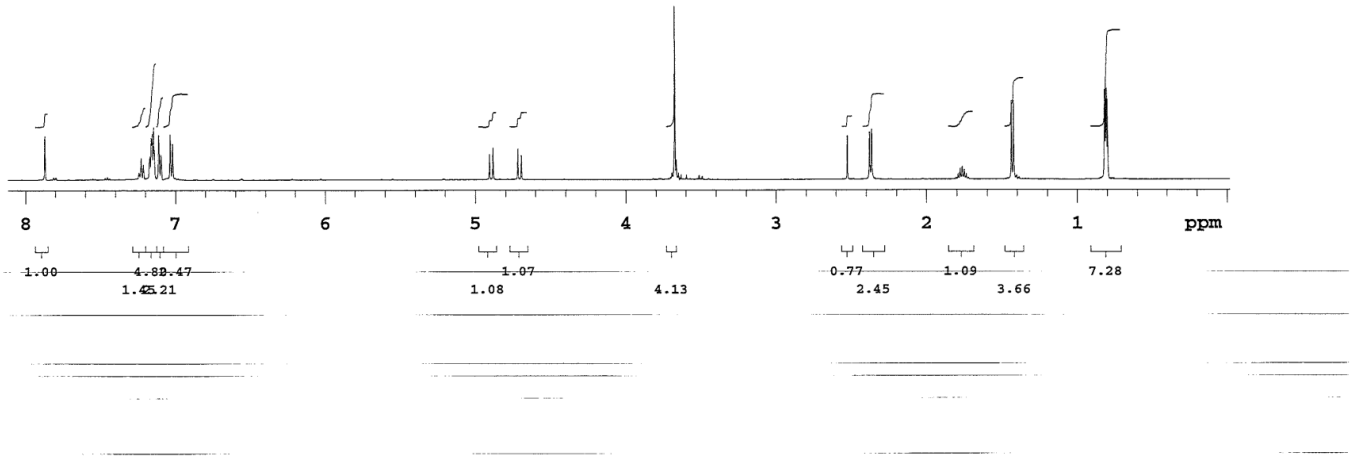
O
O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7
6
5
4
3
2
1
ppm
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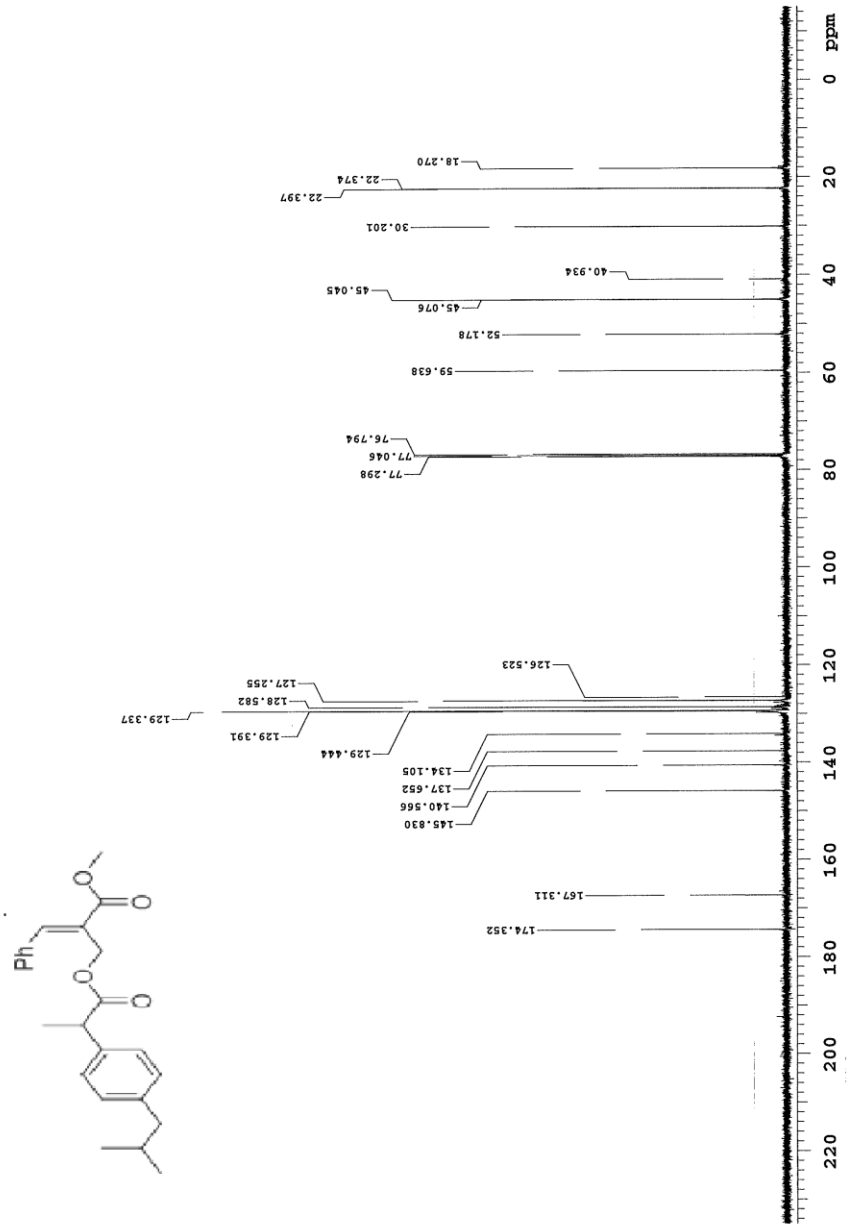
4.83
3.47
1.45
5.21
1.07
1.08
4.13
0.77
2.45
1.09
3.66
7.28


Ph
O
O
O
O
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145.830
140.566
137.652
134.105
129.444
129.391
129.337
128.582
127.255
126.523
77.298
77.046
76.794
59.638
52.178
45.076
45.045
40.934
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22.374
18.270
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80
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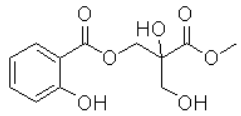
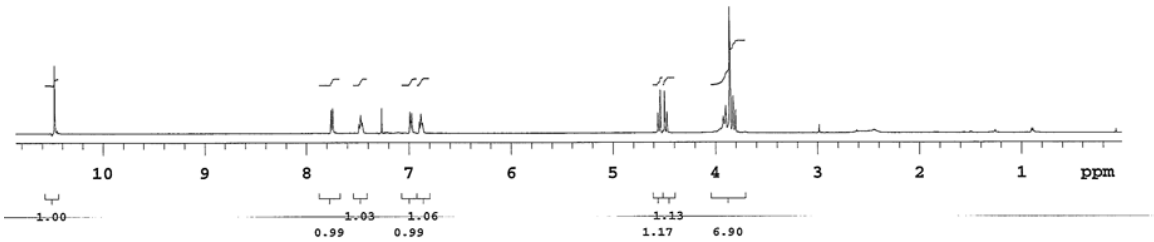
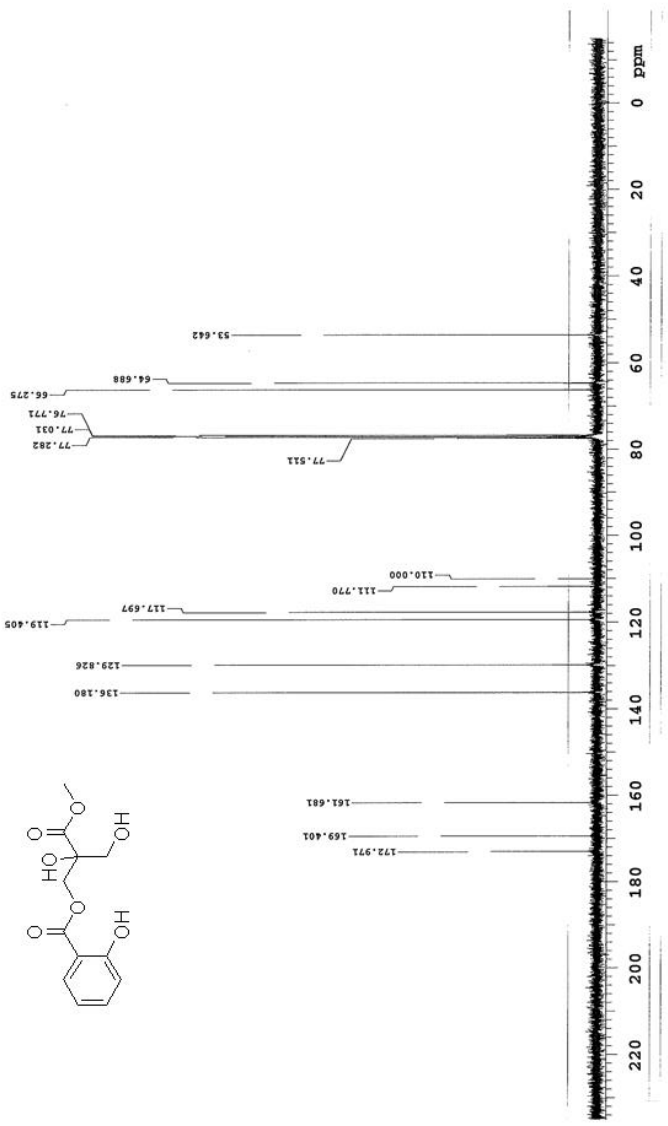
160
180
200
220


O
HO
O
O
O
OH
OH


10
9
8
7
6
5
4
3
2
1
ppm
1.00
0.99
1.03
0.99
1.06
1.17
1.13
6.90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66.275
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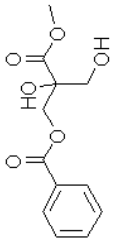
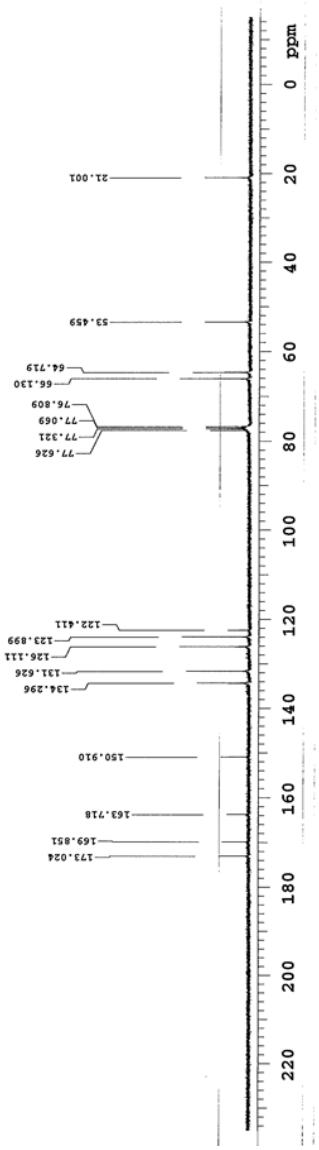
77.031
77.282
77.511
110.000
111.770
117.697
119.405
129.826
136.180
161.681
169.401
172.971
ppm
0
20
40
60
80
100
120
140
160
180
200
220
O
OH
O
O
OH
O
OH

ppm
0.97
1.14
1.03
1.00
2.20
6.69
3.57




ppm
0
20
40
60
80
100
120
140
160
180
200
220
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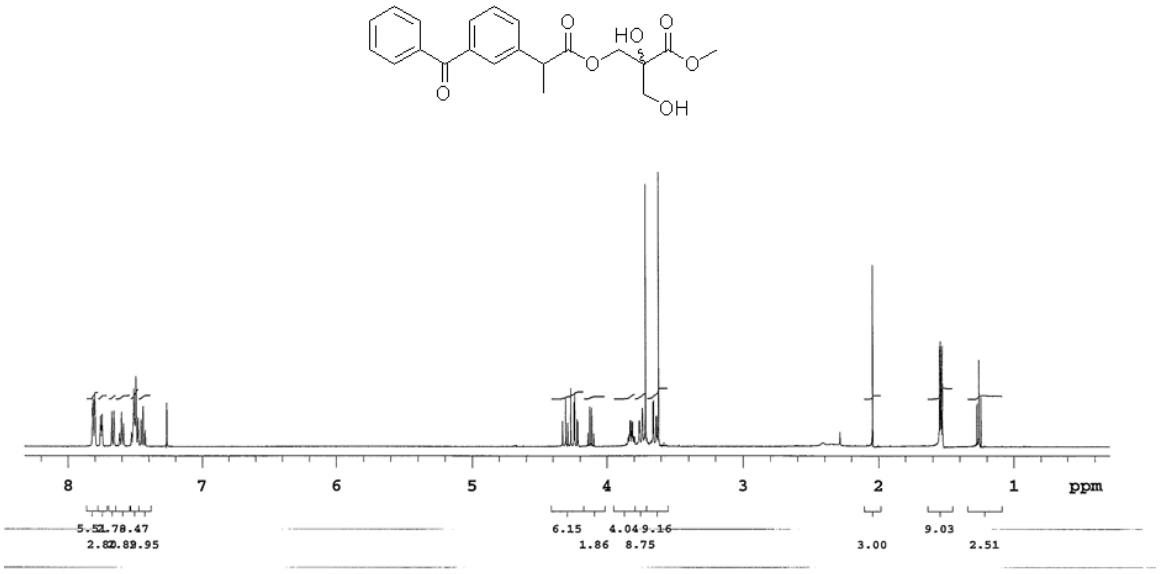
53.459
64.719
66.130
76.809
77.069
77.321
77.626
122.411
123.899
126.111
131.626
134.296
150.910
163.718
169.851
173.024


HO
O
O
O
O
O
OH
8
7
6
5
4
3
2
1
ppm
5.52
1.78
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0.89
0.95
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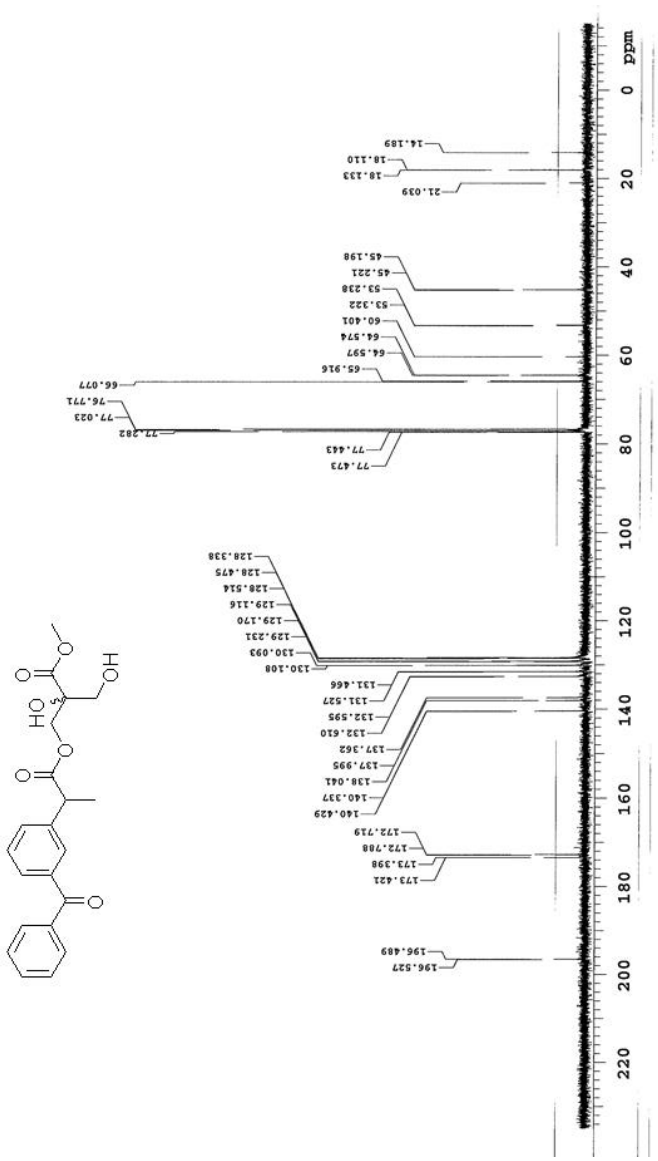
3.00
9.03
2.51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18.133
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45.221
53.238
53.322
60.401
64.574
64.597
65.916
66.077
76.771
77.023
77.282
77.443
77.473
128.338
128.475
128.514
129.116
129.170
129.231
130.093
130.108
131.466
131.527
132.595
132.610
137.362
137.995
138.041
140.337
140.429
172.749
173.398
173.421
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196.527
0 ppm
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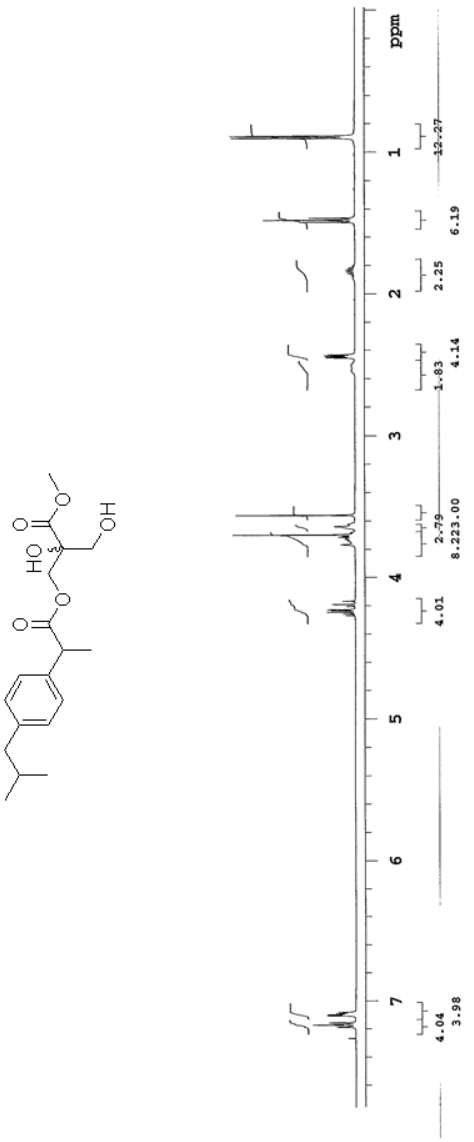
40
60
80
100
120
140
160
180
200
220
HO
OH


HO
OH
ppm
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2
3
4
5
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7
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6.19
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1.83
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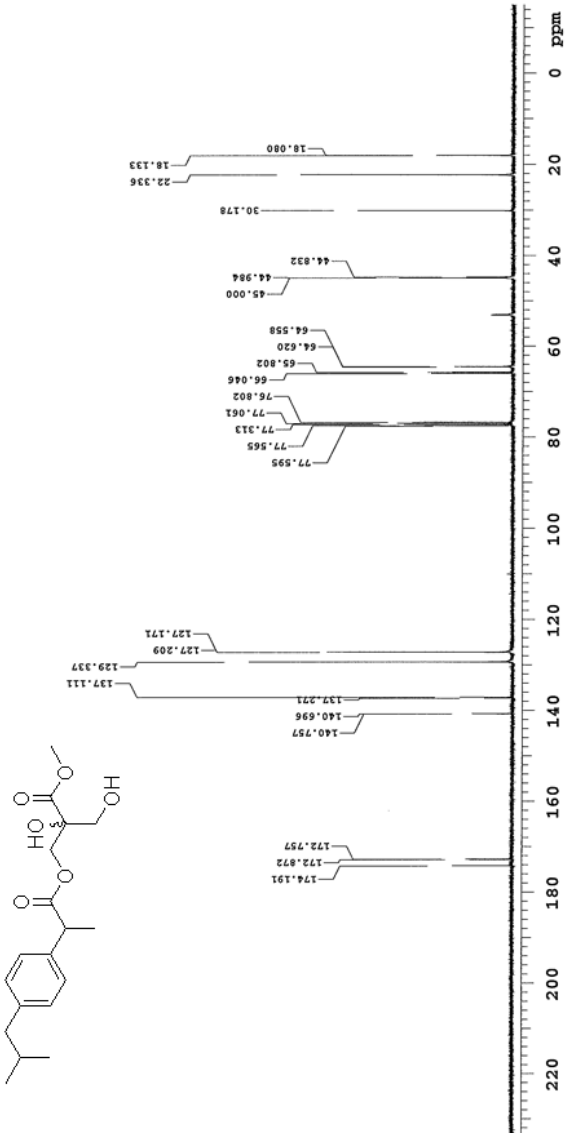
2.79
8.223.00
4.01
4.04
3.98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60
80
100
120
140
160
180
200
220
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22.336
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45.000
64.558
64.620
65.802
66.046
76.802
77.061
77.313
77.565
77.595
127.171
127.209
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174.191
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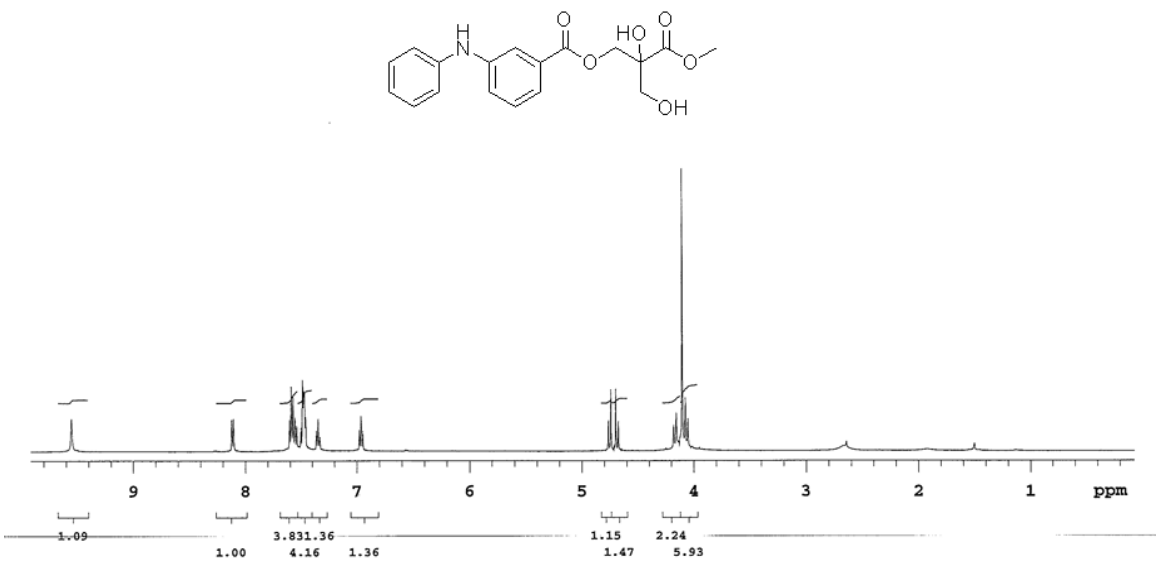
O
O
O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N
O
O
HO
O
O
OH
9
8
7
6
5
4
3
2
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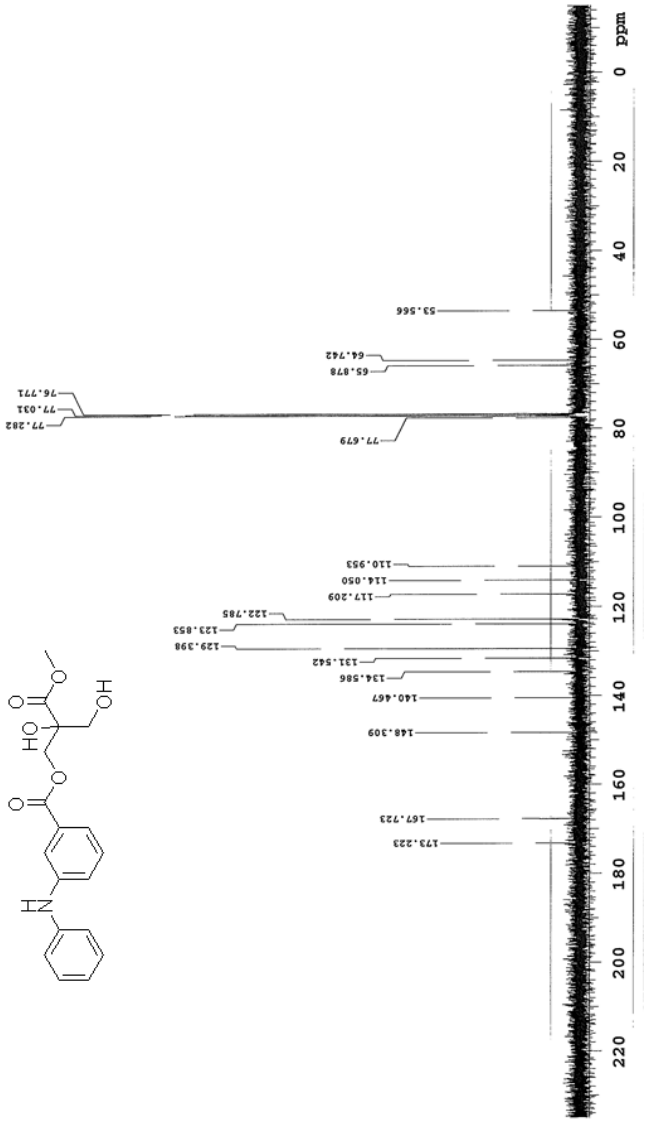
1.36
1.15
1.47
2.24
5.93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110.953
114.050
117.209
122.785
123.853
129.398
131.542
134.586
140.467
148.309
167.723
173.223
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60
80
100
120
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160
180
200
220
ppm
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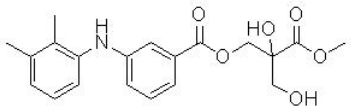
O
O
HO
NH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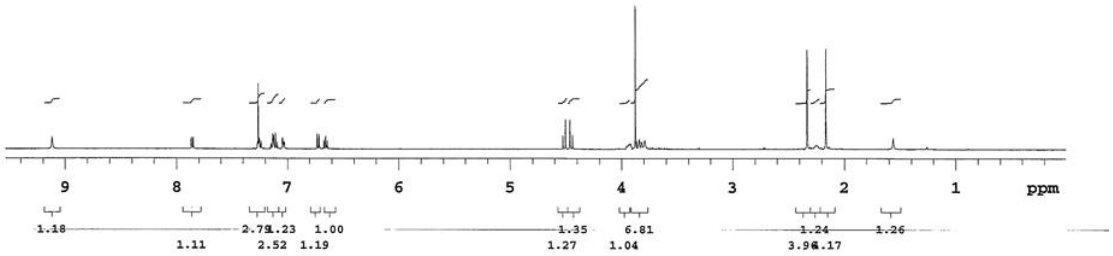
O
HO
O
O
OH


9
8
7
6
5
4
3
2
1
ppm
1.18
1.11
2.75
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1.00
1.19
1.35
1.27
6.81
1.04
1.24
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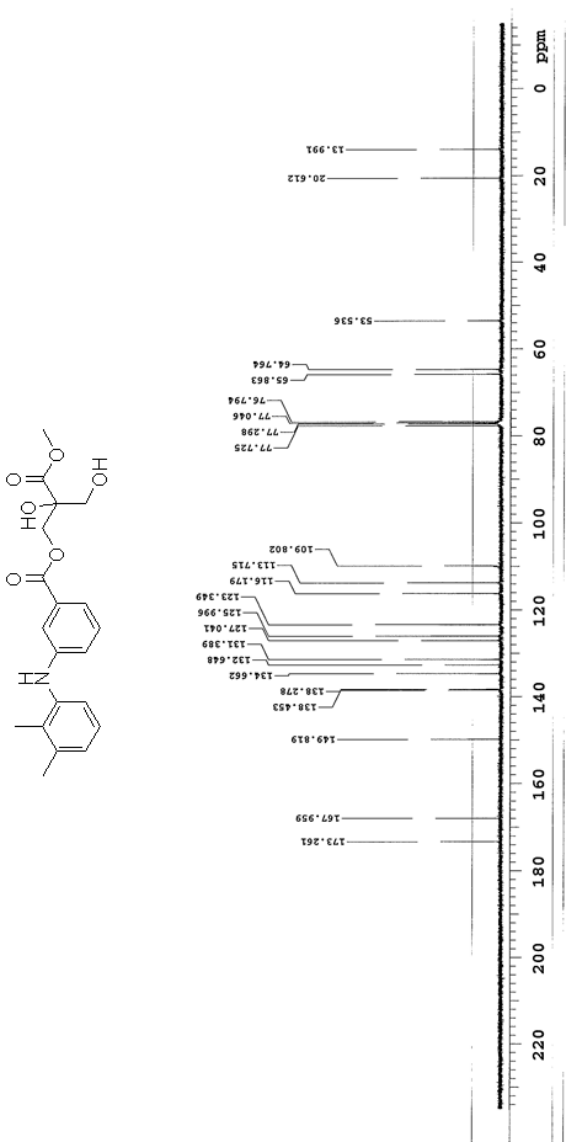
4.17
1.26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77.046
77.298
77.725
109.802
113.715
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123.349
125.996
127.041
131.389
132.648
134.662
138.278
138.453
149.819
167.959
173.261
ppm
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40
60
80
100
120
140
160
180
200
220
HO
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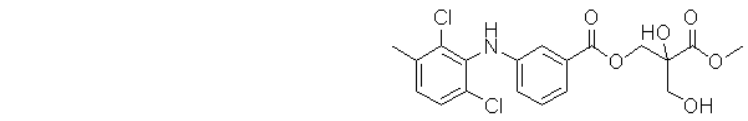
O
O
O
NH


Cl
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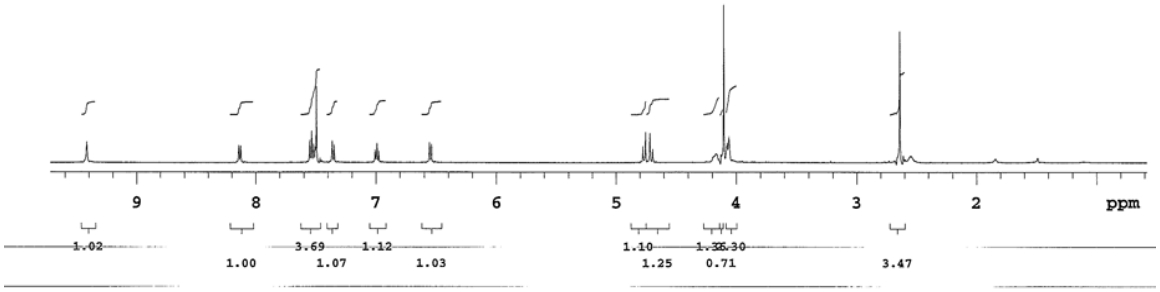
O
HO
O
O
O
Cl
OH


9
8
7
6
5
4
3
2
ppm
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1.12
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1.10
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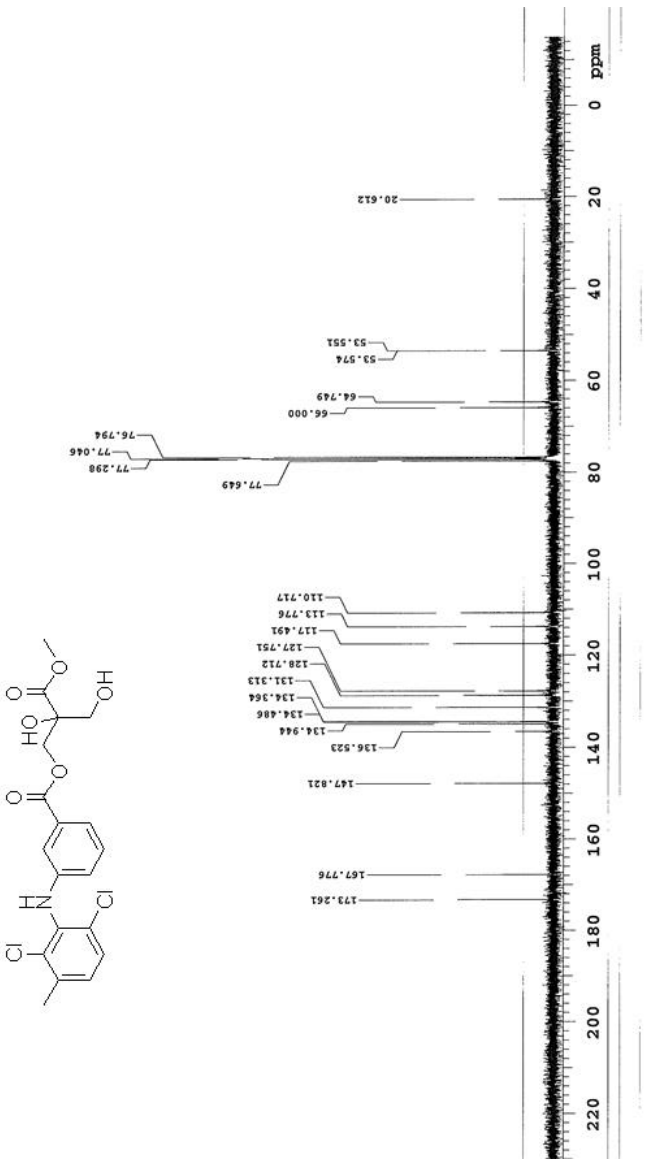
3.47


ppm
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20
40
60
80
100
120
140
160
180
200
220
20.612
53.551
53.574
64.749
66.000
76.794
77.046
77.298
77.649
110.717
113.776
117.491
127.751
128.712
131.313
134.364
134.486
134.944
136.523
147.821
167.776
173.261
O
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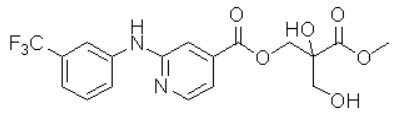
HO
OH
O
O
H
N
Cl
Cl


F3C
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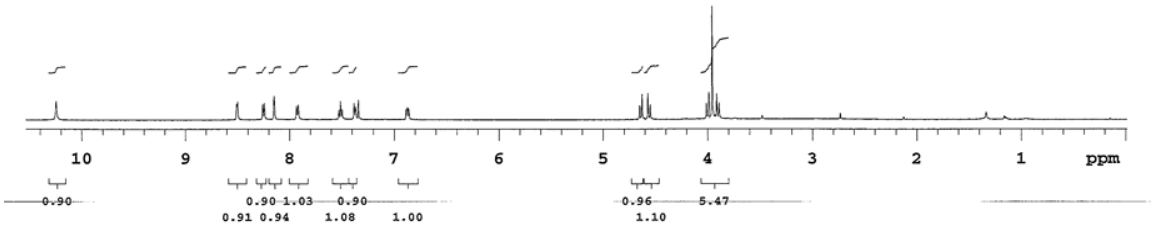
O
O
HO
O
O
OH


10
9
8
7
6
5
4
3
2
1
ppm
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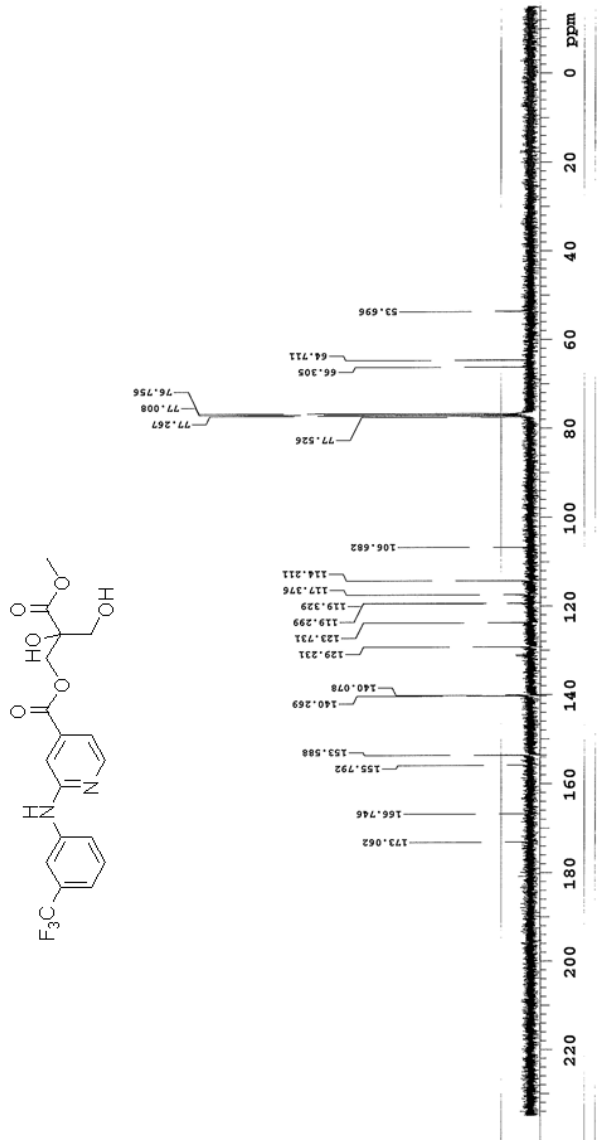
0.90 1.03
0.91 0.94
0.90
1.08
1.00
0.96
1.10
5.47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66.305
76.756
77.008
77.267
77.526
106.682
114.311
117.376
119.329
119.299
123.731
129.231
140.078
140.269
153.588
155.792
166.746
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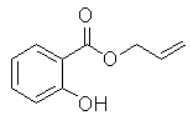
ppm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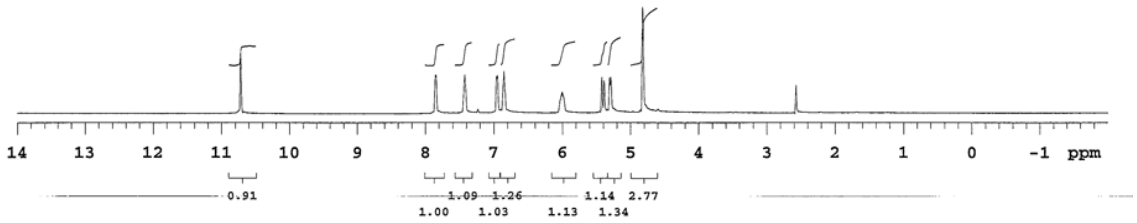
O
OH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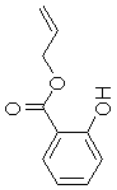
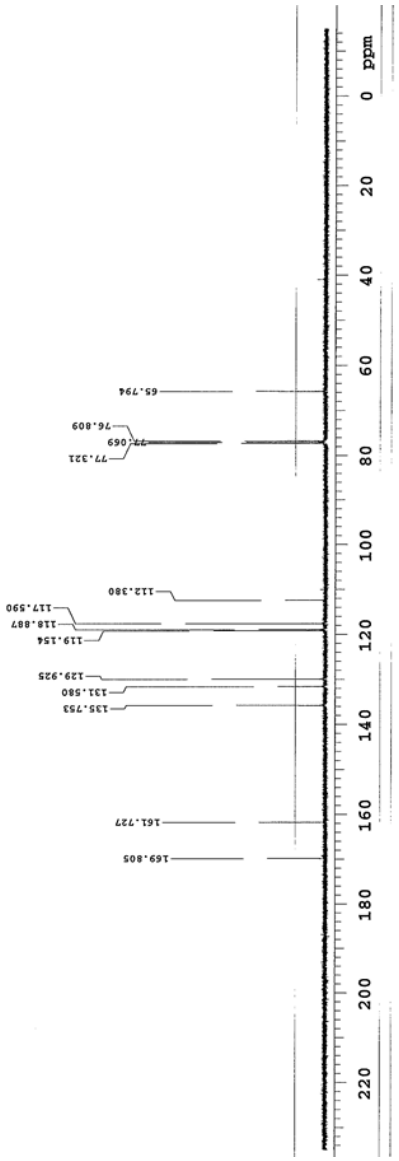
1.34

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0
20
40
60
80
100
120
140
160
180
200
220
ppm